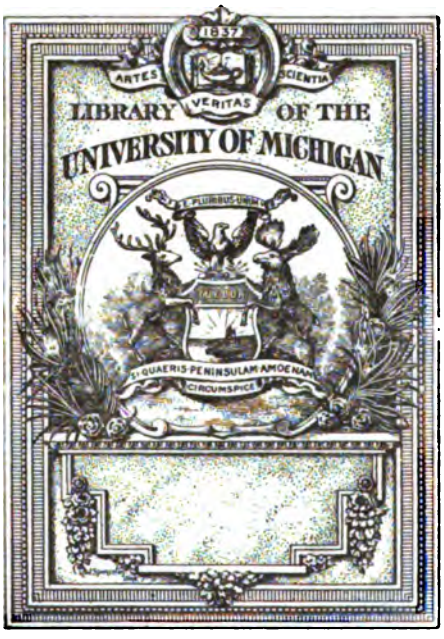
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THE EMBRYOLOGY OF THE UNIONIDAE

A STUDY IN CELL-LINEAGE

A THESIS

FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

PRESENTED TO THE

BIOLOGICAL FACULTY OF THE UNIVERSITY OF CHICAGO

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BY

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THE EMBRYOLOGY OF THE UNIONIDAE.

A STUDY IN CELL-LINEAGE.

FRANK R. LILLIE.

TABLE OF CONTENTS.

THE work recorded in the following pages was begun in the summer of 1891 at the Wood's Holl Marine Biological Laboratory, at the suggestion of Professor C. O. Whitman. It has been pursued since, subject to more or less interruption, at Clark University and the University of Chicago. In each laboratory I profited by the instruction and advice of Professor Whitman. It gives me great pleasure to acknowledge in this place my deep indebtedness to him.

I. INTRODUCTION.

The Unionidae are in many ways extremely favorable objects for embryological research, and in consequence have often been the subject of more or less careful investigation. The great ease of obtaining an almost limitless supply of material, the comparatively large size of the eggs, and the transparency of many of the larval stages make them favorable for embryological work. On the other hand, the early stages of development offer considerable technical difficulties to the student. Moreover, the extremely specialized character of the development seems to promise but little of general morphological interest.

The history of recorded observations on the embryology of the Unionidae is a long story; and as it has been rehearsed more than once by other writers on the subject (Rabl No. 25, Flemming No. 13), I need only say enough here to make my point of departure clear. A list of the early literature on the subject will be found at the end of this paper. The question

which produced such a voluminous discussion (see Part I, list of literature) was in regard to the real nature of the curious little bivalves, which were often found filling the external gills of adult Unios and Anodontas. Were they parasites or were they the young of the animals which they burdened with their incredible numbers? Rathke (No. 7, 1792) and Jacobson (No. 3, 1828) held that they were parasites of a different genus, and proposed for them the name of *Glochidium parasiticum*. The action of the Paris Academy of Sciences, which appointed a commission (No. 4) to inquire into the foundations of this curious view, is sufficient proof of the lively interest which the question excited. It would take me too far to follow the discussion in detail; it will be sufficient to say that Carus (No. 5, 1832) gave the final blow to the glochidium theory in a paper which is remarkable both for accurate observation and logical argument. Indeed, but little advance was made beyond the facts established in this paper prior to Flemming's work (Nos. 12 to 14). Contributions to the subject had been made in the meantime by Quartrefages (Nos. 23 and 24), Leuckart (No. 19), O. Schmidt (No. 20), Forel (No. 21), and van Ihering (No. 22). In 1866 Leydig (No. 20*a*) made the valuable discovery that the glochidium larva finishes its development as a parasite on fish.

Flemming's principal paper appeared in 1875 (No. 13). He gave some good figures of the maturation and early segmentation stages of the egg, and of the later larval stages; but he remained in the dark as to the passage from the one to the other, and hence came to no definite conclusion as to the origin and formation of the germinal layers. Rabl's paper (No. 25) appeared in 1876, and is in some ways a distinct advance on Flemming's. He endeavored to prove that the germ-layer theory was applicable to the Unionidae "wie bei allen Metazoen." Although he was undoubtedly right in saying that the "Vorderwulst" of Flemming was entodermic, and that the mesoderm arose from two teloblasts, yet he was just as undoubtedly wrong in his account of the origin of the entoderm as well as of the teloblasts of the mesoderm. According to Rabl, the entoderm arose as an invagination of large cells in

the region of the future dorsal surface, which freed themselves after invagination and moved to the anterior end (really to the posterior end) of the body, where they took up their definite position. Believing, as he did, that the dorsal invagination was the primitive intestine, it was natural to suppose that the teloblasts of the mesoderm, which lie just beneath the posterior end of this invagination, were derived from the cells composing its wall. There was a threefold error here: first, in mistaking the posterior for the anterior end of the larva;[1] second, in interpreting the dorsal invagination, really the shell-gland, as the archenteron; and third, in deriving the teloblasts of the mesoderm from the cells of the dorsal invagination. The first error was corrected by Schierholz (Nos. 35 and 36), and the second by Goette (No. 29). In my preliminary account I have already pointed out the true source of origin of the teloblasts of the mesoderm.

Not since the time of Rabl's paper has any work been published dealing in a thorough way with the whole embryonic development of the glochidium larva. Schierholz's paper, it is true, covers the whole period of development, but does not adduce much that is new on the embryonic portion other than the correct orientation of the larva. Goette's paper confines itself to the formation of the entoderm in Anodonta. Other papers since the time of Rabl have dealt only with the postembryonic development, thus beginning where my work ends, and hence not calling for remarks here. It would seem, then, as though it were time for another work on the embryonic development.

Great advances have been made within the last fifteen years in the adult morphology of the Lamellibranchiata; but since the time of Hatschek's paper on Teredo, no corresponding advances have been made in the embryology of these forms. In saying this I do not mean to belittle such works as those of Brooks, Jackson and Horst on the oyster or Ziegler and Stauffacher on Cyclas. During the same time many important embryological works on other classes of Mollusca have

[1] Flemming made the same mistake; Forel, who in point of time preceded both Flemming and Rabl, was right.

appeared, but, as if by common consent, the Lamellibranchiata have been ignored. The eggs of most marine lamellibranchs are exceedingly small and difficult to handle. This has probably deterred others, as it has me, from studying them, or has made the results of such studies as have appeared (*e.g.*, on the oyster) of greater economic than scientific worth.

My object in studying the Unionidae has been to fill in, so far as I could, the two gaps indicated above, *i.e.*, first, in our knowledge of the derivation of the germinal layers in the Unionidae; and, second, in our knowledge of the early development of the Lamellibranchiata. I have followed in detail the lineage of the cells as the best way of reaching conclusive results as to the origin of the germinal layers of Vermes and Mollusca. The first division of the work deals with the origin of the germinal layers. In the second part I take up the development of the gastrula in the glochidium.

II. Cleavage of the Ovum and Derivation of the Germinal Layers.

(a) *Natural History.*

The Unionidae carry their young in the external gills until the completion of embryonic development, when they are fully equipped for their temporary parasitic existence. The eggs are fertilized, after extrusion, in the suprabranchial chamber (Schierholz, No. 30, Rabl, No. 25), spermatozoa gaining access with the respiratory current of water. They then pass along the suprabranchial chamber of the internal gill to the cloaca, where they pass over to the external suprabranchial chamber, and moving along it anteriorly fill the interfoliar spaces from one end of the gill to the other.[1] In Unio the eggs adhere to one another in the form of oval plates, which may contain as many as 1000 eggs[2] each. The eggs composing each plate are bound together by a sort of cement, which gradually dis-

[1] I noticed that, in a species of Anodonta, which is very common near Worcester, Mass., only the posterior half or two-thirds of the external gills is used for the reception and development of the ova.

[2] A rough calculation.

solves out as development proceeds, finally disappearing entirely towards the close of embryonic development. The eggs of Anodonta are free from the first.

I obtained most of my material from two ponds within five or six miles of Wood's Holl. In one of these, Chiverick Pond, I obtained Unio only; in the other, Fresh Pond, Anodonta only; yet the ponds were within 500 or 600 feet of each other. The first pond, however, possessed a softer muddier bottom than the second, which was literally paved near the margin with round stones as large as cobble-stones. No doubt the different sets of conditions proved more favorable to the one genus or the other; but it can hardly be held that conditions most favorable to the one genus were necessarily fatal to the other nearly allied genus. The close proximity of the two ponds in question makes it probable that an interchange of the genera in question takes place at not infrequent intervals. Indeed, I once found a sickly-looking Anodonta in the Unio pond. It is therefore improbable that this condition was due to a difference in the original stock of the ponds in question. It seems probable that in small bodies of water, at any rate, the two genera *may* prove mutually exclusive.

An interesting difference between these two genera has been commented on by Flemming (No. 13), Schierholz (No. 30), and Carus (No. 11). This is, that, whereas all the Anodontas in a given locality extrude their eggs (which are immediately fertilized) at one or two different times, so that from a great many individuals taken at the same time only one or two different stages of development are procured, the different individuals of Unio are fertilized at different times, so that a large number taken at one time will yield several different stages of development. It is, however, noticeable in the last case that the embryos of one catch can always be grouped in five or six stages. It is thus much easier to obtain a complete developmental series of Unio than of Anodonta. For this reason, and also because the eggs of Unio are much more favorable for the study of segmentation and for sectioning, I have worked almost exclusively on this form. What I have to contribute on Anodonta concerns the glochidium only. The unsegmented

fertilized ova of Unio can be obtained from about the middle of June to the middle of July in the above locality; those of Anodonta towards the end of July and early in August.

The glochidia of Unio escape in August and September. Those of Anodonta are carried by the mother through the winter and extruded finally in the spring. Most remarkably slow is the process of cleavage in these eggs. The passage from the unsegmented egg to the 45-cell stage takes from five to seven days. I have not been able to get exact data in regard to the time of development, inasmuch as it is impossible to keep the same eggs under observation for more than a very few days. They then die, despite the utmost care. I have seen enough, however, to show the extreme slowness with which the eggs develop. Inasmuch as these ova contain but little yolk, we can only refer this slowness in development to constitutional causes. I know of no observations on the rate of development of the ova of Cyclas. On the other hand, the ova of marine lamellibranchs develop with amazing rapidity. During warm weather the young of the oyster will swim in less than twenty-four hours after fertilization.

(b) *Methods.*

The vitelline membrane is separated from the ovum by a wide space (Figs. 2 and 3, Pl. I) filled with an albuminous (or mucous) fluid, which coagulates on the addition of the usual killing reagents, making it impossible to obtain clear views of embryos mounted whole. During development the constitution of this fluid alters somewhat, so that this difficulty ceases to exist for stages later than Fig. 79 (Pl. V). I was, however, fortunate enough to find a method which gave me, at times, the most perfectly clear views of segmentation stages that could be desired; at other times it was not so successful. The embryos were exposed to the action of Perenyi's fluid for from ten to twenty minutes; they were then washed and preserved in seventy per cent alcohol. The material from which the best results were obtained remained in the alcohol for three or four months. The eggs were then mounted,

unstained, in a mixture of equal parts of glycerine and water. As the water evaporated it was replaced with pure glycerine. In the course of two or three days, in successful cases, the eggs became so beautifully transparent as to show every detail of structure with the most perfect clearness. In such preparations every nucleus, whether resting or in any of the stages of mitosis, stood out conspicuously. It was thus possible to follow all the divisions of the cells; and I can state that I have seen every spindle, up to a stage containing over fifty cells, more than once, and most of them many times.

Another method that may prove useful to others, and which I have used with success, is to kill in Perenyi's fluid and preserve the material in fifty per cent glycerine. Schneider's acetic carmine may be used for staining in this case.

For later stages Merkel's fluid is a splendid reagent. Corrosive sublimate also gave excellent results. For staining, Grenacher's borax carmine and Mayer's haemalum were used chiefly. Good whole mounts in glycerine of young larvae were obtained after the use of one tenth per cent, or even five hundredths per cent, osmic acid. To kill the glochidia with the shells open, I first added chloral hydrate to the water containing them, and, in due time, any desired killing reagent.

In all the earlier stages sections of the eggs were made *en masse*, and no difficulty was experienced in orienting sections thus made. It is a simple matter to orient the glochidium before sectioning. In hard paraffine (58° C.) the shell of the glochidium is no obstacle to sectioning.

(c) *Nomenclature.*

One of the practical questions which presents itself to the student of cell-lineage is the system of nomenclature to be adopted for the individual blastomeres. The requirements are: (1) a separate designation for each cell which will indicate its approximate location and exact ancestry in the plainest possible way, and (2) the system must be capable of indefinite expansion. These requirements sound difficult, and the fact that so far no two workers have adopted the same

system proves the difficulty to be real. Yet, if the best results are to be obtained from the comparison of the cell-lineages of different animals, some fairly uniform system of nomenclature must be adopted by the different workers in the field. For the sake of uniformity I have adopted the system followed by E. B. Wilson in his "Cell-Lineage of Nereis."[1]

The first four cells are designated by the capital letters *A*, *B*, *C*, and *D*; the generations of ectomeres by the small letters *a*, *b*, *c*, *d*; the *first* index number indicates the generation to which the ectomeres belong: thus, a^1 or $b^{1.2}$ or $c^{1.1.1}$ all belong to the first generation, c^2, $d^{2.1}$, $a^{2.2}$ belong to the second generation, and so forth. *A*, *B*, *C*, and *D* and a^1, b^1, c^1, and d^1 are retained throughout for the four vegetative and four apical pole cells, respectively. When a cell divides, the products receive the designation of the parent cell with the addition of a further index number: thus, $a^2 < \begin{matrix} a^{2.1} \\ a^{2.2} \end{matrix}$; the larger index 2 is used for the cell lying nearer the vegetative pole. Exceptions to this rule are made only in the cases of important cells, which receive special designations. Thus the first somatoblast is d^2; this designation is replaced by *X*, and the small cells formed from it by x^1, x^2, *etc.* The mesoblast is indicated by *M* for the teloblast and *m* for the smaller cells; in the case of the larval mesoblast the designation $a^{2.2}$ is replaced by *Y*. For the rest, the system will develop as the account proceeds.

Another matter which demands a preliminary explanation is the orientation given the figures of segmentation. I have oriented them all with the part, usually placed below, above. The reason for this is that it obviates the confusing changes of orientation which would otherwise be necessary. The chief part of the large (posterior) cell gives rise to the shell gland, which is dorsal; in keeping it above throughout I have had in mind the final orientation of the embryo. This is (for the same reason) the orientation which has been used by other writers on the Unionidae. At the close of the work there is inserted a section on the change of axes.

[1] The system of nomenclature lately proposed by Mr. Kofoid (No. 49 *a*) is too rigidly symmetrical to be applicable to a cleavage so irregular as that of Unio.

(d) *Cleavage.*

1. THE FIRST TWO FURROWS.

The unsegmented ovum adheres to the vitelline membrane only in the region of the micropyle. The polar globules are invariably formed just opposite to this point. This fact has been commented upon by other authors and I have satisfied myself with merely observing it. The significance of the fact seems, however, to have escaped notice. The micropyle marks the point of detachment from and, in earlier stages, the point of attachment to, the ovarian wall (*cf.* Stauffacher, No. 59). We can thus trace back the orientation of the ovum to the earliest stages of its development in the ovary. The polar globules cannot form at any point, but must form at *one* point in this almost alecithal ovum. The animal and vegetative poles, and therefore the relations of the ectoderm and entoderm are (normally) determined *before the ovum leaves the ovary.* The importance of this fact in the interpretation of cleavage is sufficiently obvious.

The first segmentation plane divides the ovum into two unequal portions *AB* and *CD* (Pl. I, Fig. 4). The division runs from the animal to the vegetative poles and is inclined at an angle of 45° to the future longitudinal and transverse axes of the embryo. (Text — Fig. 1.) When first fully formed the two cells are round and meet over a comparatively small area (Fig. 4); but they at once begin to press against each other and to flatten at the point of contact (Fig. 5). This process may go on until the whole egg has assumed again nearly the form of a sphere; the only external indication of the separation of the two parts being a shallow constriction where they meet. But no actual fusion of the cells ever takes place, for in section there is always a sharp line of division. The smaller cell *AB* is clearer than the larger cell *CD*, as both Rabl and Flemming have remarked; but this difference in appearance is to be ascribed rather to its smaller size and hence greater transparency, than to any marked histological differentiation.

Each cell contains entoderm as well as ectoderm, hence Rabl's designation of animal cell (*AB*) and vegetative cell (*CD*) is inapplicable (No. 25).

The second plane of division is likewise meridional, and practically at right angles to the first. The two cells divide at different times, and these two divisions taken together represent what is usually called the second furrow in other eggs. In the two-cell stage then there arises a certain independence in the times of cleavage of the blastomeres. Later when one cell gains a start, so to speak, over the corresponding cell of another quadrant, it continues to maintain or may even increase its lead. This difference in the time relations of the divisions of the cells of different quadrants is one of the most striking features in the cleavage of Unio. The four cells of a quadrant never divide synchronously; the difference in time may be slight but it remains constant throughout many divisions.

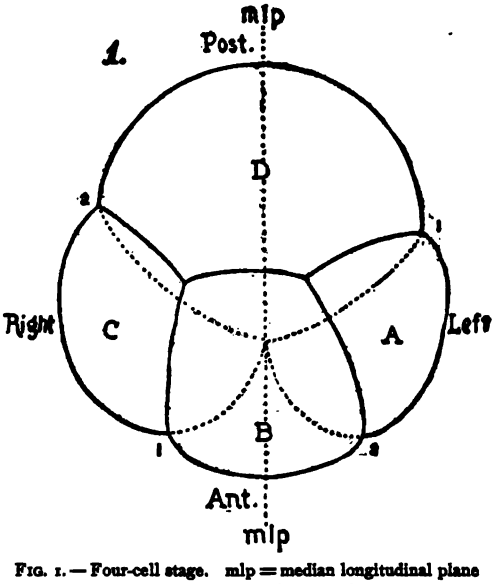


FIG. 1. — Four-cell stage. mlp = median longitudinal plane of larva. 1—1 = First cleavage-plane. 2—2 = Second cleavage-plane.

CD is the first to divide; the products of division are unequal (Pl. I, *C* and *D*, Figs. 6 to 9). The smaller cell *C* lies on the right side of the future embryo; *D* is much larger and occupies the posterior end of the embryo. During the division of *CD*, *AB* begins to constrict (Pl. I, Fig. 6), and shortly after the separation of *C* and *D* divides into two approximately equal parts (Pl. I, Figs. 8 and 9). Of the four cells now making up the embryo *A*, *B*, and *C* are approximately equal; *B* is as a rule slightly smaller than *A* or *C*; *D* is very much larger than any of the other three. (Text — Fig. 1.)

This early division of *CD* is the first indication of a tendency to progressively more rapid cell multiplication in the posterior

part of the embryo, which leads at last to fundamental disturbances of the axial relationships of the parts.

Figs. 9 and 10 (Pl. I) show two different phases of the four-cell stage. In the earlier phase (Fig. 9) *B* and *D* meet over a small area at the apical pole, the usual Brechungslinie or cross-furrow of authors. In the later phase (Fig. 10) *B* and *D* meet over a wide area. There is now an extensive cross-furrow at the animal pole. *A* and *C* meet over a very small area at the vegetative pole. In most forms, *e.g.*, Clepsine, Nereis, Crepidula, if either cross-furrow be the larger, it is that at the vegetative pole. The difference finds its explanation in the fact, that the greater mass of the first four blastomeres is ectodermal in Unio and entodermal in the above mentioned forms. The special character of the cross-furrow is hence due to the accumulation of the specific plasms at their own poles of the egg. E. B. Wilson comes to practically the same conclusion when he says that the reduction of the apical cross-furrow as compared with that at the vegetative pole in mollusks and annelids "stands in obvious relation to the different size of the cells produced at the two poles."

The orientation of the embryo is now a matter of no difficulty. The large posterior cell *D* maintains its much larger size in relation to the other cells of the embryo. Its more numerous divisions, moreover, give a most characteristic and unmistakable appearance to the posterior end of the egg.

Previous authors have failed completely to understand the significance of these first four blastomeres. Rabl held that *A*, *B*, and *C* were ectomeres, and that *D* formed all of the entoderm and mesoderm as well as some ectoderm. Flemming expressed no opinion on the subject, and Schierholz followed Rabl. As a matter of fact, each of the four cells contains both ectoderm and entoderm. The mesoderm is formed later from *D*, but in a totally different way from what Rabl thought, as will be shown later. In addition to ectoderm and entoderm *A* forms a special kind of mesoblast, for which I shall retain the term larval mesoblast, which I used in my preliminary paper.

2. The First Generation of Ectomeres.

Shortly after the division of *AB* a small cell d^1 is budded off from *D* towards the apical pole. We have thus a five-cell stage which has been figured by Rabl and others. The three other cells, *A*, *B*, *C*, next bud forth small cells a^1, b^1, and c^1 likewise towards the apical pole. These three divisions do not take place synchronously, but in the almost invariable order *C*, *A*, and *B*, so that six, seven, and eight-cell stages occur. The four apical cells thus formed are the first generation of ectomeres.

Fig. 13 is a view of the apical pole in the six-cell stage. *D* and *C* have already divided, and it will be seen that both *A* and *B* are preparing to divide. In both of these cells the aster to the left belongs to the ectomere; the one to the right is sunk deep in the substance of the cell, and hence is represented as being fainter. This figure shows further that the oblique nature of the cleavage is already indicated by the spindles. Thus the spindle in *B* points to the space between *B* and *C*, that in *A* to the space between *A* and *B*. Fig. 14 from the vegetative pole shows the time relationships of the cleavage very well; c^1 is already separated from *C*, though the asters are still visible in both cells; the metakinetic stage of division has been reached in *A*, while *B* is in the equatorial-plate stage. Fig. 15 is a view from the apical pole of the completed eight-cell stage. It will be seen at once that d^1 is the largest of the ectomeres. Fig. 16 is a view of the same stage from the right side, and partly from the vegetative pole.

Fig. 12 illustrates an interesting condition which occurs but rarely. The four macromeres are of more nearly equal size than usual; in this case *A* is larger than *D*. This may, perhaps, be a reversion towards a more primitive type in which the segmentation was equal. It may not be out of place to mention here that all of the eggs obtained from one individual of Unio complanata divided equally at the start. As it was impossible to keep the eggs alive long enough, I cannot say that normal embryos would have been produced. But from the vigor with which the early cleavages took place I feel convinced that such would have been the case under normal conditions. Watasé (No. 60) has

also noticed that "eggs from the same animal show similar variations in cleavage"; he concludes that "such a tendency to vary may become hereditary."

The first generation of ectomeres occupy a position in relation to the macromeres which might be supposed to be reached by their rotation after formation through an angle of 45° in the direction of the hands of a watch. This arrangement is obviously well adapted to economize space; if it were produced by an actual rotation of the cells after their formation there would be no difficulty in understanding it. But the fact that the cleavage spindles are oblique, even before the lobing of the cytoplasm, shows that the process is not a purely mechanical one, in the sense that it is produced by the mutual pressure of the blastomeres. This manner of division is characteristic of the ovum of molluscs (cephalopods excepted), annelids and polyclads. Selenka (No. 58*a*) was one of the first authors to describe it. Speaking of the four ectomeres, Selenka says that they are budded off from the four macromeres "im Sinne einer laeotropen oder λ-*Spirale*." Blochmann (No. 35), who was the next to describe this type of cleavage, in a prosobranch, Neritina, used a different method of expression; he said that, looked at from the animal pole, the first generation of ectomeres during their formation underwent a shifting "im Sinne des Uhrzeigers," and the second generation "in der Uhrzeigerbewegung entgegengesetzten Sinne." Lang (No. 53) used the same form of expression as Selenka; but neither of these authors has made use of the term spiral to distinguish a special type of cleavage. Wilson, on the other hand, has characterized this form of cleavage as the "spiral" type in distinction from his "radial" and "bilateral" types. Heymons (No. 47) seems to prefer Blochmann's comparison with the hands of a watch, for he simply refers to the other expression as "sog. spiralige." Kofoid (No. 49*a*) uses the term spiral in the same way as Wilson. It seems, then, that there have been two ways of describing this form of cleavage: first, by the use of the term spiral, and second, as a rotation.

Wilson (No. 65, p. 600) derives the "spiral" from the "radial" form of cleavage by "a twisting of the radii, as it were, the

blastomeres being displaced or rotated with respect to the egg-axis, either to the right, following the hands of a watch (right-handed spiral), or in the reverse direction (left-handed spiral), as the case may be." "The term 'spiral' refers to the fact that the curved radii, if prolonged, would form a spiral about the egg-axis." In the ontogeny there is no twisting of the radii, but merely an inclination of the axis of the dividing cell from the vertical. It seems to me, therefore, that this form of cleavage would be more correctly termed *oblique.*

In the radial type cleavages are either vertical or horizontal with respect to the egg-axis; the cleavage spindles are hence, respectively, horizontal or vertical. In the oblique type the second cleavage spindle is not horizontal, but oblique. From the point of view of an observer in the axis of the egg, the spindle is inclined from right below to left above, which can be expressed by the single word leiotropic. In the third cleavage the spindle is dexiotropic. Regarding the ovum from the animal pole, the upper cell lies to the left of the lower, *i.e.*, in a leiotropic position, in the first instance, and to the right, *i.e.*, in a dexiotropic position, in the second instance. The second cleavage of the macromeres is leiotropic, and the third dexiotropic again. In the following pages the cleavages will be described as leiotropic or dexiotropic according as the inclination of the cleavage spindle from below above is to the left or right of the vertical axis of the ovum, and not according to the direction of the actual planes of division.

Crampton (No. 41*a*) has discovered that in Physa, a sinistral pulmonate, the directions of the cleavages are reversed. Thus, the spindle in passing from the four to the eight-cell stage is leiotropic, not dexiotropic as in the other cases cited. Inasmuch as the obliquity changes from right to left, or *vice versa*, with each successive cleavage, as in the other cases, the mesoblast is formed from the *right*, not the *left*, posterior macromere. The direction of the division of the left posterior macromere at the fourth cleavage would throw its product on the left side of the embryo, while the homonymous product of the right posterior macromere is thrown in the middle line behind. It would seem in this case that the position of the

fourth product determined whether or not it is to be mesoblast; but that this conclusion is not justified is shown by the fact (which Mr. Crampton very kindly permits me to add) that the macromere which is to form the mesoblast divides before the other. Its prospective function is structurally outlined before this cleavage. For the origin of this reversed mode of cleavage we must go back to the two-cell stage. The cleavage from two to four cells is leiotropic in Limnaea, and dexiotropic in Physa; on this depends the localization of the mesoblast in the first case in the left, in the second in the right posterior macromere. Some structural difference of the two ova conditions the primary difference in the direction of the cleavage, on which all subsequent variations depend.

This is the first time,[1] as already stated (No. 21), that an eight-cell stage has been found to exist in the lamellibranchs resembling the form so characteristic for this stage in all other Mollusca (Dentalium excepted), with holoblastic ova, as well as in Annelida and Polyclada, not to mention numerous other forms. This stage, in its typical form, consists of four micromeres lying upon and alternating with four macromeres, the former being derived one from each of the latter, and lying to the right of the parent macromere. It is true that Flemming describes the origin of the eight-cell stage in Anodonta in the same way as I have done, but he does not figure it clearly. He says (p. 130, *l.c.*): "Nachdem der Keim in dem Stadium der Fig. 10" (four-cell stage) "wieder länger mindestens mehrere Stunden geruht hat, beginnt der nächste Act der Theilungsarbeit, und zwar wieder in analogen Weise wie der letzte: auch jetzt theilt sich der dunkle Obertheil (*i.e.*, *D*) in zwei ungleich grosse Segmente, andererseits proliferirt der jetzt dreizellige Untertheil: nur laufen diese Processe hier

[1] Korschelt and Heider state in their "Lehrbuch" that Lankester described for Pisidium an eight-cell stage formed of two superimposed layers of four cells each, and they refer the reader to his well-known article (No. 54). Any one who will take the trouble to read the first two pages of his work and to look at his Fig. 17, will find that Lankester described *four* meridional furrows before the appearance of the "first circumferential," or equatorial furrow. Thus the eight-cell stage would consist of eight cells in one plane. This is undoubtedly an error of observation. It is, however, different from the account of Korschelt and Heider.

nicht gleichzeitig nebeneinander ab," *etc.* He then describes in more detail the origin of d^1 (5 of Flemming) exactly as I have done, and goes on to say (p. 131, *l.c.*): "Inzwischen schicken auch Zelle 2, 3 und 4 (*C*, *B* and *A*) zur weiter Theilung an, so aber dass diese erst nach der Produktion von 5 (d^1) und nicht bei allen drei Zellen gleichzeitig erfolgt." I am very glad to find my statement of these simple and easily-observed facts in such exact agreement with Flemming; the more so, as I am forced to differ from Rabl, who describes d^1 as dividing immediately after its formation, a thing in itself but little probable.

I am the more particular in emphasizing these facts, as Korschelt and Heider, in the third part of their "Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere," make the following statements about the cleavage of the lamellibranch ovum: "Die Furchung stimmt bei denjenigen Formen bei welchen sie genau untersucht wurde (Unio, Anodonta Cardium, Cyclas, Teredo) in so auffallender Weise überein, dass man auf einen ähnlichen Verlauf derselben auch bei den Formen schliessen darf von denen einzelne ähnliche Stadien bekannt geworden sind (Ostrea edulis, Pecten, Mytilus edulis). . . . Die Furchung ist stets inäqual. . . . Die kleine Furchungskugel theilt sich in zwei und ungefähr gleichzeitig oder wenig später schnürt sich von der grossen eine neue Furchungskugel. Auch diese theilt sich und sodann wiederholt sich derselbe Vorgang der Abschnürung eines Mikromers von der grossen Furchungskugel. Ganz der gleiche Process findet nochmals statt, indem von der grossen Zelle kleinere geliefert werden, welche sich sodann theilen. Die Mikromeren sitzen schliesslich, von der Oberfläche gesehen, wie eine Kappe auf der grossen Furchungskugel, welcher letzere erst später sich in zwei Zellen theilt."

It is hardly necessary for me to state that I cannot accept this as a general scheme applicable to all lamellibranchs. If one looks over the literature on lamellibranch embryology, one is struck by nothing so much as the meagerness of the details on cleavage. It has generally been thought sufficient to describe it as far as the six or seven-cell stage and to say:

"the same thing is repeated and results in the formation of a cap of small cells lying on a large one." The large cell is held to be entodermal always, but in Unio I am certain that it is not so.

It seems impossible at present to reduce the two hitherto described forms of cleavage in lamellibranchs to a common law; but Korschelt and Heider compare Rabl's account of the cleavage of Unio with Hatschek's account of Teredo, in spite of the fact that the first cleavage of the larger cell is meridional in the first case and equatorial in the second, according to the description given. According to Ziegler and Stauffacher, the cleavage of Cyclas is like that of Teredo; the latter's sections are convincing; one can no longer doubt that the first cleavage of the larger cell is equatorial in this form. How this type of cleavage can be derived from the oblique type, it would be difficult to say. But perhaps the only difference is that the obliquity of the spindle is greater.

3. FROM THE EIGHT TO THE SEVENTEEN-CELL STAGE.

A nine-cell stage is formed in Unio complanata. This stage is reached from the eight-cell stage by a division of the posterior macromere *D* in an equatorial plane (Pl. II, Figs. 17 and 18). The division is unequal, the ectomere d^2 being much larger than the macromere[1] *D*. d^2 is the "first somatoblast" (v. Wistinghausen) and in succeeding stages will be designated by the letter *X*. It is also the first of the second generation of ectomeres. The formation of the other members of the second generation of ectomeres is illustrated in Fig. 19, Pl. II (a view from the right side); a^2, b^2, and c^2 are larger than their parent macromeres *A*, *B*, *C* (Pl. II, Figs. 20 and 21). The cleavage-spindles of the second generation of ectomeres are leiotropic.

d^1 divides next, thus producing the thirteen-cell stage (Pl. II, Fig. 20, apical pole). The divisions of the other members of the first generation of ectomeres soon follow. The order of

[1] The term "macromere" will be retained throughout for the four hypotropic cells (Goette) without reference to their relative size.

the division is c^1, a^1, and b^1, thus repeating the order of the first divisions of *D*, *C*, *A*, and *B*.[1] Figs. 22 and 23 (Pl. II) illustrate the appearance of the apical and vegetative poles respectively in the seventeen-cell stage. The seventeenth cell is x^1 (Fig. 23), which has been budded forth from *X* just behind *C* on the vegetative pole. (Cf. Figs. 20 and 24 of Nereis; Wilson *l.c.*) The first division of the first generation of ectomeres is leiotropic.

4. From the Seventeen to the Thirty-eight-cell Stage: Third Generation of Ectomeres, etc.

The eighteen-cell stage is reached by another division of *D* (Pl. II, Fig. 23). The cell to the left is the first member of the third generation of ectomeres (d^3, Figs. 25 and 27). A period of rest now ensues. When activity is again resumed, spindles appear almost simultaneously in a^2, b^2, c^2, and *X* (d^2). The positions of these spindles and, in consequence, of the resulting cells is very different in the different cases. Figs. 24, 25, and 27 (Pl. II) illustrate the description. A cell, x^2, is budded off from *X* on the left side, symmetrical with x^1 on the right side and just posterior to d^3 (Pl. III, x^2, Fig. 29). The cleavage of c^2 and b^2 is equal and dexiotropic. The division of a^2 calls for special attention, inasmuch as the larval mesoblast is separated by it. Fig. 25 shows that the division will be equatorial. After the completion of the division it is seen that the cell nearer the vegetative pole (Pl. III, $a^{2.2}$ or *Y*, Fig. 29), which becomes the larval mesoblast, is the larger. *Y* is bounded by the following cells: on the right by x^2 and d^3, on the left by $d^{1.1}$ and $a^{2.1}$, behind by *X*, and in front by *A* and part of *D*. Already (Pl. III, Fig. 29) it is partially covered by x^2 and d^3.

During these divisions the cells of the apical pole lose their rounded contour and together form a flat plate of cells (Pl. II, Fig. 26). They now enter upon a long period of rest, during which extremely important changes, which lead to the establishment of the mesoblast and entomeres, take place at the

[1] Lang has observed that in Discocoelis the first generation of micromeres follow the same rhythm of division as their parent macromeres (No. 53, p. 331).

vegetative pole. Before passing on to a description of these changes, I will direct attention to another division of *X* (Pl. III, Figs. 31 and 33), by which a small cell is budded off towards the apical pole just posterior to $d^{1.2}$ (Pl. III, x^2, Fig. 35). The appearance of this cell at this time and place is extremely interesting, seeing that it tallies exactly with Nereis (E. B. Wilson *l.c.*, Pl. XVI, Fig. 33).

The remaining members, a^3, b^3, and c^3, of the third group of ectomeres are now formed by the simultaneous leiotropic division of *A*, *B*, and *C* (Pl. III, Figs. 32 and 34). After these divisions the entoderm is definitely localized in the four cells *A*, *B*, *C*, and *D* (Pl. III, Fig. 38). The larger part of *D* is, however, mesoderm, which in the next figure (39) is shown about to be definitely separated. *When this division is completed (Pl. IV, Figs. 41 and 42) the delimitation of the germinal layers in distinct blastomeres is accomplished.* The other spindles in Fig. 39 explain themselves, and the resulting cells are shown in Fig. 42.

The embryo at the time of the separation of the germ-layers contains thirty-two cells (see table of cleavages, p. 33). At the same time the embryo of Nereis contains thirty-eight cells. The difference is due to the suppression of cleavage in the apical pole cells of Unio. The composition of the embryo can be gathered from the following table:

Entomeres. $A-D$	4
Mesoblast. M (d^4)	1
Larval Mesoblast. Y and y^2	2
Ectomeres of first generation	10
Ectomeres of second generation ($b^{2.1}$, $b^{2.2}$, $c^{2.1}$, $c^{2.2}$ and $a^{2.1}$)	5
First Somatoblast	6
Ectomeres of third generation	4
	32

The number (32) of cells at this stage is a chance coincidence merely; it has not been reached by a geometrical progression from two, four, eight, sixteen, to thirty-two cells, as in a synchronously cleaving ovum.

Almost every cell in this stage has so distinctive a character that if isolated from the cell-complex it could be recognized

by its general form. The embryo has the appearance of an irregular mosaic.

After the establishment of the germ-layers in separate cells, and before the beginning of bilateral divisions, a fourth division of the first somatoblast takes place. A small cell (x^4, Fig. 39) is budded off anteriorly towards the vegetative pole and against the posterior end of the second somatoblast. This division of *X* does not occur in this form in Nereis. The fourth division in Nereis is equal and bilateral, whereas in Unio the fifth is the first bilateral division. Other divisions take place at about this time on or in the region of the vegetative pole, which give it a most characteristic appearance. These are the divisions of x^1, d^3 and *Y*. x^1 buds off a small cell towards the antero-lateral border of the second somatoblast ($x^{1.1}$ and x^1 Figs. 39 and 40). A division of exactly the same general form takes place in Nereis (No. 64, Figs. 52 to 54). d^3 divides somewhat later ($d^{3.1}$ and $d^{3.2}$, Figs. 40, 42, and 45; $d^{3.2}$ helps in the overgrowth of *M*). This division of d^3 is interesting from the fact that the other micromeres of the third generation do not divide till much later. d^3 has apparently inherited the tendency of its parent macromere *D* to rapid division. *Y* buds off y^1, between d^3, *A* and a^3 (Pl. IV, Figs. 39 and 40), and a little later y^2, on the other side (Pl. V, Fig. 59).

While dealing with these divisions it will be just as well to include the observations which I have made on the further divisions of the first and second groups of micromeres. If the order of division were the order of description it would be necessary to postpone this for some time later, but in that case, I fear that the reader would be as tangled up in the description as I was at one time in the apparently confused and indeterminate order of the facts. I have followed the first group of ectomeres to a stage when sixteen cells of this group are formed. It would have required much time and trouble to have followed them farther: and, inasmuch as no larval apical organs are formed, I desisted. I have not, therefore, found the cross on which Conklin lays so much stress in Crepidula; I doubt very much its existence in any stage in Unio, for I should certainly have seen it, were it formed.

I have no doubt that there is a causal relation between the rudimentary condition of the pre-velar region and the slow and irregular character of the cleavages of the first generation of ectomeres which forms this region.

d^1 is generally the first of the apical pole cells to divide. The products are d^1 and $d^{1.2}$, which abuts against x^3. c^1 generally follows, and $d^{1.1}$ about the same time. Then comes $c^{1.1}$ and a^1, followed by $a^{1.1}$; b^1 and $b^{1.1}$ are the last of the ectomeres of the first generation to divide. After their division a spindle appears in $d^{1.2}$. Fig. 51 exhibits an unusually regular arrangement of these sixteen apical cells. It can easily be seen there that, while the first division of the four central cells a^1, b^1, c^1, and d^1 was leiotropic, the second division is dexiotropic.

We have already noticed one division of the three anterior members of the second generation of ectomeres. This division was obliquely equatorial in the cases of b^2 and c^2, and much more nearly horizontal in the case of a^2. In this latter case the variation is correlated with the formation of the larval mesoblast. We shall treat the divisions of d^2 (the first somatoblast) and $a^{2.2}$ (the larval mesoblast) separately, and so have now to concern ourselves simply with $b^{2.1}$, $b^{2.2}$, $c^{2.1}$, $c^{2.2}$, and $a^{2.1}$. $b^{2.2}$ and $c^{2.2}$ lie nearer the lower pole than $b^{2.1}$ and $c^{2.1}$; $b^{2.1}$ and $b^{2.2}$ are of approximately equal size; the same is true of $c^{2.1}$ and $c^{2.2}$. These four cells divide almost simultaneously, though $c^{2.1}$ leads the other three by a little. The plane is in each case nearly horizontal (Figs. 41, 42, 43, 45, and 47); each cell divides somewhat unequally: in the cases of $b^{2.2}$ and $c^{2.2}$ the smaller product lies nearer the vegetative pole. The reverse happens with $b^{2.1}$ and $c^{2.1}$; $b^{2.2.2}$ comes to lie in the angle enclosed by a^3, A, B, and b^3. This group of cells has a very characteristic appearance, as shown in the figures (*e.g.*, Figs. 42 and 43). Fig. 43 is an anterior view of the egg, and shows very clearly the divisions of $b^{2.1}$, $b^{2.2}$, and $c^{2.2}$. The spindle of division of $c^{2.1.2}$, which I have several times seen, is shown in Figs. 45 and 47. As for $a^{2.1}$, a single division of this cell is represented in Figs. 48 and 49, and the two resulting cells are shown in Figs. 50 and 51.

What is the fate of the second generation of ectomeres? The answer will be given separately for d^2 and $a^{2.2}$; the others form the larval mantle, or, at least, contribute to its formation. This being so, we have a satisfactory explanation of their large size, which is due to the precocious segregation of this large and important organ in single cells. The text figures on p. 59 show the relation of these blastomeres to the future embryonic areas.

5. Establishment of Bilateral Symmetry. The Larval Mesoblast.

The spindle of bilateral division of the first somatoblast is seen in Fig. 44 (Pl. IV). This is a view from behind of a stage slightly older than Fig. 42 (Pl. IV). Shortly after the completion of the division indicated, each of the resulting cells buds forth a small cell x^5 towards the vegetative pole (Pl. IV, Figs. 45, 46, and 47). These two small cells are placed just behind $x^{1.1}$ and x^4 (Pl. IV, Fig. 45) *and form with the cells* x^1, $x^{1.1}$, x^2, *and* x^4 *the beginning of a tongue of cells, which grows forward and over the second somatoblast. Between the entomeres and the two cells X, X (the protoblasts of the shell-gland) there are no cells after the inclusion of the mesoblasts but the derivatives* (x^1–x^5) *of the first somatoblast.* Therefore there is no room for doubt, that, after the invagination of the entomeres and inclusion of the mesoblasts, all of the cells lying between the blastopore and the posterior end of the shell-gland are derivatives of the first somatoblast. I anticipate here what will be shown in detail later, *viz.:* that these cells form the ciliated plate (Wimperschild of Flemming) from which the foot is derived later on.

The next bilateral division is that of the second somatoblast (mesoderm proteloblast) *M* (Pl. IV, Fig. 45). Fig. 46 shows the two mesoblasts just after the completion of this division. The invagination of the entoderm is slightly indicated in this figure, and its position is sufficient proof that the cells concerned are *A*, *B*, *C*, and *D*.

With these two divisions (*i.e.*, of the first and second somatoblasts) the bilateral character of the cleavage becomes apparent. By this I do not mean to say that there is a complete cessation

of oblique divisions; on the contrary the micromeres of the first generation continue for some time to divide obliquely and the same holds true for other cells. But from now on there is no difficulty in recognizing the fact that we are dealing with a bilateral embryo, and little by little all parts of the embryo are brought into relations of bilateral symmetry.

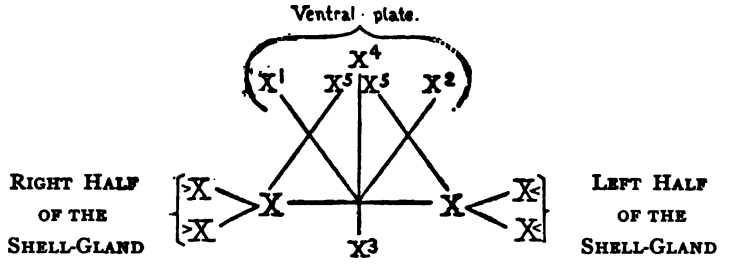
There is no more striking instance of this than in the behavior of the larval mesoblast *Y*, which during these changes has been more and more overgrown by the surrounding cells (Pl. V, Fig. 59). It has budded off two small cells y^1 and y^2 (Pls. III, Fig. 39 and V, Fig. 59), and I am inclined to think, though I am not certain of it, that a third small cell is budded from *Y*, before the latter enters the segmentation cavity. Fig. 61 is an optical frontal section of a stage with four large dorsal cells, products of *X*; the larval mesoblast is here seen to lie almost entirely within the segmentation cavity. Fig. 62, an actual transverse section of a still later stage, shows *Y* in the process of equal bilateral division. *Thus, though in origin asymmetrical, the larval mesoblast comes, apparently by active migration, to be placed symmetrically in later stages.* In Fig. 62 the next important change in the history of the larval mesoblast is shown. Each half is dividing. The resulting cells are shown in section in Fig. 66 (Pl. V). In some of my preparations I have seen spindles in the two cells of the larval mesoblast, which would lead to the formation of extremely small cells lying against the ventral wall of the embryo near the region where the oral plate later appears. In yet others I have seen these cells fully formed. I am not certain that this always takes place, but when it does it always precedes the division just mentioned.

6. THE FIRST SOMATOBLAST.

To return to the cells *X*, *X*, from which the shell-gland is formed. Fig. 60 shows the right cell already divided into fairly equal parts and the left in process of division. The order of division of these two cells is invariably as figured; the two divisions are never synchronous, but I have not been able to correlate this difference of time relations with any

future difference of the two sides of the shell-gland. The four resulting cells are seen faintly outlined in Fig. 61, which also shows that the two anterior cells are somewhat the smaller. By repeated divisions a plate of high columnar cells is formed (Pl. V, Figs. 64, 66, and 72), which occupies the whole of the future dorsal region of the larva. It has caused an enormous expansion of the area previously occupied by the cell *X* (*cf.* Pl. V, Figs. 59 and 72), and has thus completely altered the embryonic topography (*vide* text figures, p. 59), ***establishing the permanent dorsal axis of the larva and adult.***

Let us tabulate the divisions of the first somatoblast:



The cells referred to the center of the bar joining the two central *X*'s, are those which were budded from the first somatoblast before its bilateral cleavage. The table indicates that the first somatoblast is the protoblast of two very important organs, *viz.:* the shell-gland and the ventral plate.[1]

I cannot forbear calling attention again to the wonderful similarity of the cleavages of the first somatoblast in Nereis. (*Cf.* table on p. 410 of E. B. Wilson's Nereis work.) In Unio the first somatoblast buds forth four small cells, whereas in Nereis it buds forth three small cells only, before its bilateral cleavage; the place of formation and relative size of the cells is exactly the same as in Unio. One would expect from its original position that it would form the mid-dorsal region in Nereis as in Unio; and as a matter of fact such seems to be

[1] I use the term "ventral plate" with full knowledge of its significance in annelid embryology. The term is, however, so applicable to this tongue of cells, and the fate of the cells in question is so similar in part to those of the Annelid ventral plate that I feel justified in using the term.

the case. However, according to Wilson, "*the residual teloblasts move apart so as to leave a triangular space between them covered with small transparent cells* (*dor.*) *and at the same time they gradually recede from the prototroch towards the lower pole.* This change in position of the teloblasts is of fundamental importance, since I believe the triangular area to represent the middle dorsal region of the adult body, and the residual teloblasts to mark the posterior limit of the ventral plate." (The italics are Wilson's.) This quotation alone would give one the impression that the middle dorsal region was not formed from the first somatoblast. However, later on, p. 419, Wilson says that "the small cells which separate the posterior teloblasts from the prototroch" are "the descendants of x^3 and (?) of x^6, x^6. *From the latter, as I believe, arise the cells that occupy the triangular area between the two residual teloblasts after their divergence. This area afterwards forms the middle dorsal area of the trunk.*" Thus in Nereis as in Unio the median dorsal as well as the ventral surface is a product of the first somatoblast. There is in Nereis, but not in Unio, a latero-dorsal strip of the trunk epiblast which is derived from a^2 and c^2. In Nereis the formation of the ventral surface from the first somatoblast is unmistakable, the origin of the dorsal surface is not so apparent. In Unio the reverse is the case; thus at first sight it seems as though there were a reversal of surfaces, so that cytogenetically the dorsal surface in Unio would correspond to the ventral surface in Nereis; but that such is not really the case is sufficiently apparent on a closer analysis.

I am under obligations to my friend, Mr. A. D. Mead, for permission to record his observations on this subject. Mr. Mead has found in Amphitrite, one of the tubicolous polychaeta, *that the collective ectoderm of the trunk, dorsal and lateral as well as ventral, is formed from the first somatoblast.* "*The small transparent cells*" ("*dorsal cells*" *of Wilson*) *form the dorsal ectoderm of the head segment only.* Unless Nereis differs radically from Amphitrite, Wilson has been in error in regard not to the origin but to the fate of the dorsal cells.

7. The Second Somatoblast.

We have already become acquainted with the lineage of this cell; in the last section we saw further that its first division was equal and bilateral. It will now be in place to trace the further history of the two resulting cells up to the time of their inclusion within the segmentation cavity as the mesoderm teloblasts. Their position is immediately behind the entomeres (Pl. IV, Fig. 46). In this stage the products of the first somatoblast are beginning to overgrow them.

The next division of the mesomeres (Pl. V, Fig. 60) is peculiar. It is very unequal, and the two small cells *m*, *m*, are budded forth just on the posterior lip of the blastopore. This tallies exactly with Nereis. In Nereis these superficial divisions are continued for some time, but in Unio such is not the case. Soon afterwards the mesomeres are included within the segmentation cavity, and take up their definitive position behind the archenteron (Pl. V, Figs. 66 and 67). v. Wistinghausen has observed that in Nereis dumerilii about one half of the second somatoblast remains within the bounds of the ectoderm as the "untere Urzellen des Rumpfes," and forms the anterior part of the ventral plate.

According to Stauffacher's recent observations (No. 59) on Cyclas cornea, two cells placed symmetrically on either side of the middle line divide in such a way that about one half of each cell comes to lie in the segmentation cavity. According to Stauffacher the two cells in the segmentation cavity are the protoblasts of the mesoderm; the two cells on the surface the protoblasts of the entoderm. It is to be observed, however, that the last point is a pure assumption. The cells in question cannot be, or, at least, have not been, traced into the entoderm; and there seems to be a total lack of points of orientation for fixing on the region of these cells, and of the later entoderm cells as identical. I am inclined to think that the surface cells correspond to the cells budded off on the surface by the mesoblast in Nereis and Unio, and that the entomeres lie in front of this point in the form of a plate of relatively small cells. This view, at any rate, accords more

nearly with what we know of mesoderm formation elsewhere; whereas Stauffacher's account stands alone.

Wilson describes the cells which have been budded off at the surface by the primary mesoblast as forming a pigment area, and later wandering within the segmentation cavity as "secondary mesoblast." The significance of this fact in the interpretation of mesoblast cells of "ectodermal origin" can hardly be overestimated. I believe that the same ultimate fate awaits the two superficial mesoblast cells in Unio. In Fig. 67 it will be seen that between the most posterior cell of the primitive intestine and the mesoderm teloblast there lies a small cell, which is at the most anterior end of the ventral plate, the exact position of the cells *m*, *m*. It may be that they are actually these cells, but it is impossible to prove it. In their present position it would be easy for them to be pushed within the segmentation cavity.

After the teloblasts of the mesoderm have entered the segmentation cavity, each buds forth anteriorly a small cell (Pl. V, Figs. 63, 66, and 67). This is the beginning of a mesoblastic germ band on each side. The two bands are parallel, and grow forward in contact in the median line just beneath the large cells of the shell-gland. The teloblasts are forced within the segmentation cavity by the forward growth of the tongue of cells derived from the first somatoblast. The progress of their inclusion can be traced through a series of figures (45, 46, *etc.*). $x^{1.1}$, x^4, and x^5–x^5 are very active factors in the process.

(e) *Gastrulation and Shell-Gland.*

The archenteron is derived from the entomeres *A*, *B*, *C*, and *D*. Before they invaginate to form the primitive intestine they increase considerably in number (Pl. V, Fig. 60). Even before this stage is reached a slight indentation is noticeable in the region of the entomeres. The invagination deepens, and soon forms a small sac communicating freely with the exterior (Figs. 65, 66, and 67). Fig. 65 is a view of this stage from the ventral surface (the entoderm is colored in sepia). Fig. 64 is a view from the dorsal surface, and Fig. 72, from

the side. These views are given in order to make the external topography of this important and hitherto misunderstood stage clear. In Fig. 72 the reference line running to *Y* passes through the blastopore region. The position of the larval and primary mesoblast[1] are other points of orientation which make comparison with Figs. 64 and 65 easy.

Figs. 66 and 67 (Pl. V) are two successive sagittal sections through an embryo of this stage. Fig. 67 is a true median section, while 66 passes a little to one side of the middle line. The dimensions of the entodermic sac are comparatively insignificant, as one would expect from the slight initial size of the entomeres. In this stage the blastopore has a considerable antero-posterior extent (Fig. 67).

It will be noticed that the primary mesoblast lies behind, and the larval mesoblast in front of the primitive intestine. There is thus no possible chance of confusing the two structures. They are as distinct in appearance and position as in origin. It was indeed this stage which first convinced me of the twofold origin of the mesoblast in Unio. By following the clue back I arrived at the results already given.

Previous observers have completely overlooked this stage, or, at least, its anatomy. Goette is the only one who has given a correct account (which is, however, very incomplete) of the origin of the entoderm in the Unionidae, his observations having been made on Anodonta. While he is no doubt right in holding that the invagination of the entoderm occurs at the spot indicated; still my observations both on whole and sectioned larvae of Unio lead me to think that an invagination would be found in an earlier stage of Anodonta at the region in question.

If I take up the shell-gland now, it is because of its having been more than once (Rabl, Schierholz) confused with the primitive intestine; also because I have been able to ascertain its cytogeny with certainty, which gives it a place in the first, or cytogenetic division of the paper. The cytogeny of this organ has been already described, so that we can begin

[1] I use the term primary mesoblast for all mesoblast derived from the teloblasts *MM.*

here with the stage of Figs. 64, 66, 67, and 72 (Pl. V), where the gland is represented by a plate of large cells occupying the whole dorsal[1] region. These cells invaginate, and so give rise to the shell-gland, the long axis of which is transverse to the long axis of the embryo (Pl. V, Figs. 69 and 70); it is of an enormous size as compared with other molluscan embryos. This is, of course, a special provision for the needs of the glochidium, which possesses an enormous shell in proportion to its bulk. So large is this gland that its invagination makes an appreciable difference in the diameter of the embryo, as may be seen by a comparison of Figs. 66 and 69, both of which are camera drawings with the same lenses. It might be said that Fig. 69 was drawn from a smaller embryo; but, as a matter of fact, embryos of this stage are always smaller than in the stage just before the invagination of the shell-gland. A large shell-gland seems to be characteristic of lamellibranch embryos. Reference to figures and comparison with the embryos of other Mollusca will illustrate my point.

It is not my intention to dwell on the view which interpreted this gland as the archenteron. That is not, I suppose, any longer held by any one. But it is rather remarkable that two observers, Rabl and Schierholz, should have seen the gland migrate bodily to the region in front of the ventral plate. I can only suggest that the observations were made on partially macerated embryos, in which I have myself seen appearances which might deceive in some such way.

(f) *Summary.*

The first cleavage is unequal; the second divides the smaller cell equally and the larger unequally. The four-cell stage is composed of three subequal and smaller cells and one large cell, which lies at the posterior end. One of the smaller cells is anterior and the other two right and left, respectively. The ectoderm is separated from these four cells in a series of three

[1] The term dorsal here refers to the adult, and not the embryonic axis; the question of axial relations is entered into further on.

oblique cleavages, the first of which is dexiotropic, the second leiotropic, and the third dexiotropic again. The next division (*i.e.*, the fourth) of the posterior macromere separates the proteloblasts of the mesoderm.

The first generation of ectomeres divides very slowly. Its cells are destined for the anterior end of the future larva.

The second generation of ectomeres is remarkable for being composed of the largest cells in the embryo. The posterior member d^2 or X is the protoblast of the shell-gland and ventral plate. The larger part of the left member (*i.e.*, $a^{2.2}$ or Y) forms that portion of the mesoblast which I have called the larval mesoblast. $a^{2.1}$, b^2, and c^2 enter into the formation of the larval mantle. The third generation of ectomeres probably does the same.

The entomeres are small. They undergo division before invagination. The resulting archenteron is extremely small, *but it invaginates before the shell-gland;* thus, though rudimentary, its formation is not delayed.

The shell-gland is a voluminous structure formed from a plate of large columnar cells, which are derived from repeated divisions of X.

The first bilateral cleavage is that of the first somatoblast; the second, that of the second somatoblast.

The primary mesoblasts enter the segmentation cavity and lie just behind the archenteron, thus in the angle which the latter makes with the shell-gland. Their divisions are typically teloblastic. It should be said that, before they enter the segmentation cavity, each buds off a small cell at the surface. The larval mesoblast conforms to the bilaterality of the embryo after entering the segmentation cavity. Its divisions are not teloblastic.

The table of cleavages which follows gives, in some detail, the order of the divisions and the destiny of the blastomeres. The stages indicated by columns separated by continuous lines represent, more or less nearly, natural periods of rest of the entire ovum. The vertical columns united by dotted lines indicate more or less synchronous divisions. This method was adopted to show clearly the very irregular course of the

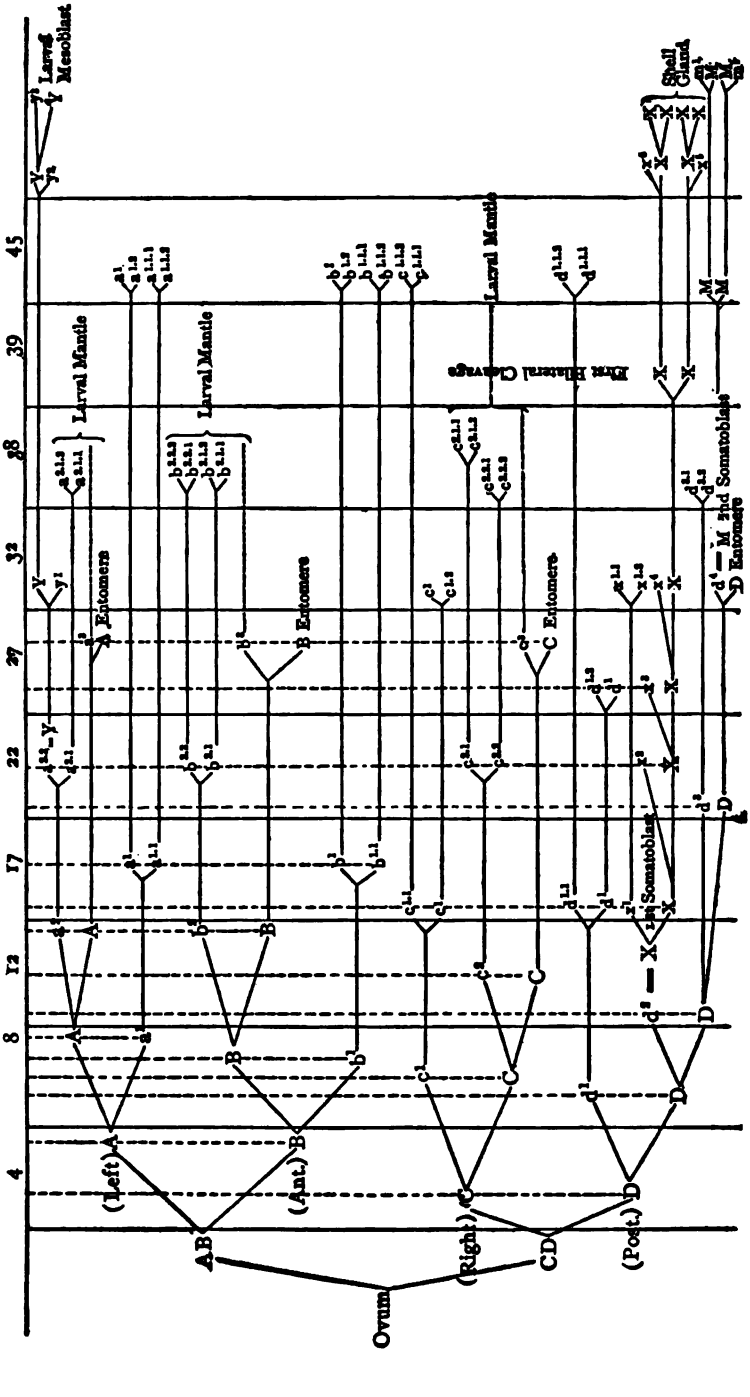
cleavages. For a general survey the dotted vertical lines can be left out of account, and the cleavages within one column thought of as synchronous. I include in an appendix the results of some of the most important works on cytogeny reduced to tabular form. To make comparisons more simple all systems of nomenclature have been reduced to the one employed here; but the original system has, in most cases, been included in brackets, after the common system.

(g) *General Remarks on the Cleavage and Germ-layers.*

Increasing accuracy and detail in the study of the cleavage of the ovum has been one of the most marked tendencies of recent years in embryology. Primarily undertaken, in the Invertebrata at least, to explain conflicting statements as to the origin of the germinal layers in general and the mesoblast in particular, the study of the cleavage is leading to new ideas on the promorphological state of the ovum, and the real nature of differentiation. In the Polyclada, Annelida, and Mollusca, not only the germinal layers, but systems of organs, and even single organs have been traced back to their parent blastomeres, and it has been shown that cells of the same lineage, even in widely separated species, undergo, as a rule, the same ultimate differentiation.

The mesoblast was the first of the germ-layers to be traced back to a single cell. Kowalevsky (No. 52) in 1871 showed that the mesoblast of Lumbricus could be traced back to two posterior pole cells, derived from the entoderm, which budded off anteriorly, a large number of small cells thus forming a mesoblastic germ band. He postulated a double source for the mesoderm of Euaxes: the larger part came from two large cells derived from the posterior macromere; the rest was derived from two small cells, derivatives of the two lateral macromeres. Rabl (No. 25) formulated his results in 1876 as follows: "Das mittlere Keimblatt entsteht also nach unseren Auseinandersetzungen aus zwei, am Mundrande der Gastrula gelegenen Zellen, deren Verwandschaft zu den Zellen des inneren Blattes eine viel innigere ist, als zu jenen des äus-

TABLE OF CLEAVAGES.



seren." The origin of the mesoblast, from two bilaterally symmetrical cells, has often since been described. Professor Whitman (No. 61, 1878) was the first to show that there was a perfectly definite cell history and origin from single cells for other structures of the adult. He demonstrated that, in Clepsine, not only the mesoblast, but the ventral nerve cord and the trunk nephridia could be traced back to single cells; and further, that the cells representing these structures were the product of the posterior macromere of the four-cell stage, which was thus the representative in this stage of the whole trunk. In 1879 Rabl's paper on Planorbis appeared. He showed, for the first time, that the whole ectoderm was formed in a series of three cleavages each, from four basal macromeres. Blochmann (No. 35), 1882, described three generations of ectomeres in Neritina, and derived the mesoblast from the fourth cleavage of the posterior macromere. During the last twelve years a great many papers dealing with the cleavage have appeared. In this section I wish to point out the most obvious results of the later work on the annelids and molluscs, and to show the bearing of my observations on Unio.

As I have already said, Rabl was the first to show for any form that the epiblast is formed in three generations of ectomeres from four basal macromeres. (Fol described these three generations, but believed that the macromeres contributed to the epiblast after that.) Since then the same thing has been shown to be true for Neritina (Blochmann, No. 35), Umbrella, (Heymons, No. 47), Crepidula (Conklin, Nos. 39 and 40), Limax (Kofoid), and Unio among the mollusca; and for Nereis limbata (E. B. Wilson, No. 64), Nereis dumerilii (v. Wistinghausen, No. 66), Polymnia, Spio, and Aricia (E. B. Wilson, No. 64) and Amphitrite (Mead) among the Annelids.[1] v. Wistinghausen

[1] McMurrich (No. 56) thinks that more than three generations of micromeres are formed in Fulgur. He thinks that "probably the amount of yolk present influences the number of spherules formed." It may, of course, be true that more than three generations of micromeres are formed in Fulgur, just as in Polymnia and Aricia (Wilson, No. 64, p. 458). The important point to determine is how many of these generations are ectomeres. From the recent studies it seems to follow that while the number of generations of *micromeres* is variable, the number of generations

described the mesoblast as formed by the third cleavage of the posterior macromere. E. B. Wilson has already pointed out his probable error. In all of the other cases cited the mesoblast is formed by the fourth cleavage of the posterior or left posterior macromere. Heymons (No. 47, p. 271) has already emphasized this fact.

The accompanying diagrams will serve to emphasize the facts already dwelt on and to bring out others. The first diagram illustrates one point of fundamental importance, *viz.:* That the posterior macromere contains all the elements of the trunk in Clepsine Nereis,[1] Amphitrite, and Unio. The first somatoblast (neuro-nephroblast, Whitman) forms the ventral plate and all ectomermal elements of the trunk; while the second somatoblast forms the mesodermal elements of the trunk. (In no other mollusc (Diagram 2) has the ectoderm of the trunk been traced to the posterior ectomere of the second generation. But from an inspection of Heymons' and Conklin's figures I have but little doubt that the shell-gland at least is derived from this blastomere; the cytogeny of the foot is not as yet known in these forms.) It is probable, too, that in all these cases the members of the first generation of ectomeres form the region in front of the prototroch. This has been shown beyond doubt for Nereis (two sp.) Umbrella, Crepidula Unio, and Amphitrite, and made more than merely probable for other forms. In all cases, too, the second and third generations of ectomeres probably take up homologous positions in the body. The macromeres after the separation of the ectomeres and mesoderm (by the same number of cleavages) become entomeres.

of *ectomeres* is invariable (Wilson, No. 64; Heymons, No. 47) in Annelida and Mollusca; all micromeres after the third generation, with the exception of the posterior micromere of the fourth generation (mesoblast), being entodermic. From Brobetzky's description of Nassa one would conclude that more than three generations of micromeres are formed; but until it is shown that all are ectomeres, we cannot accept this as evidence against the views upheld here. Similarly it is not certain as yet just how many generations of ectomeres are formed in Clepsine. However, it has not yet been shown for any annelidan or molluscan ovum of the oblique type of cleavage that more or less than three generations of ectomeres is formed.

[1] In Nereis a dorsolateral strip of trunk epiblast is derived from *A* and *C*, according to Wilson.

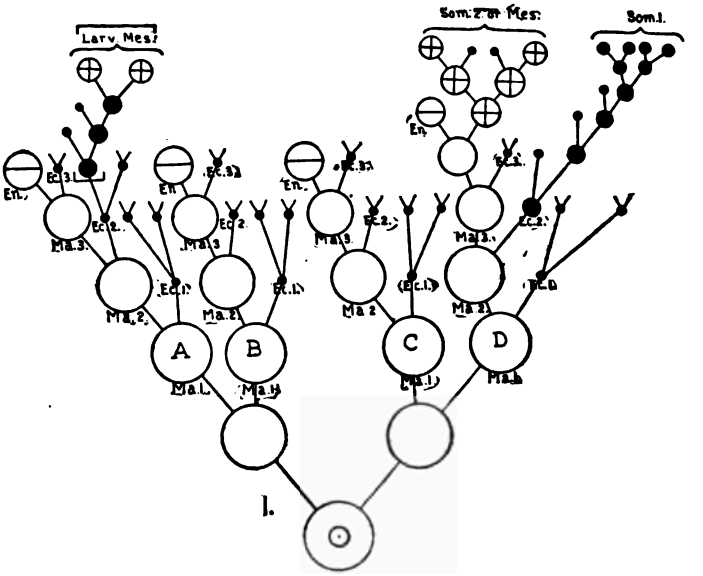


FIG. 1. — Diagram of the cleavage in Clepsine, Nereis, Amphitrite, and Unio. The part in square brackets is peculiar to Unio. In Clepsine the third generation of ectomeres has not been found and the mesoderm is formed from the posterior ma. 3. — ec. 1., ec. 2., ec. 3., first, second, and third generations of ectomeres. ma. 1. to ma. 4., Macromeres of different stages. en., Entomere. Larv. mes., Larval mesoblast. som. 1., First somatoblast. som. 2., Second somatoblast.

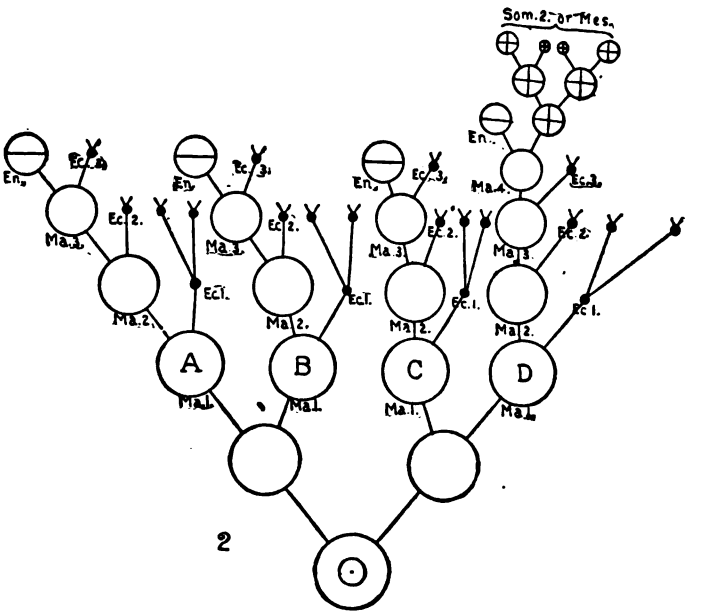


FIG. 2. — Diagram of the cleavage in Crepidula, Umbrella, Neritina, Planorbis, Limax, and Physa.

It is thus possible to speak of an homology of cells. Does it therefore follow that all cells of the same lineage in these different forms are homologous and their products likewise, and that we must deny the homology of cells which, although of different lineage, yet in the end produce homologous organs? I think not. Yet it may be said that if we can speak of homology in the one case, why not in the other? To which it may be answered, that the only safe test of homology *in such cases* is the end result. We have these facts: (1) Cells of the same lineage have the same fate in a wide series of forms (*e.g.*, mesoblasts = fourth cleavage of *D*. (*Cf.* also supra.) (2) Cells of the same lineage have a different fate in forms which otherwise agree closely (*e.g.* $a^{2.2}$ in Nereis and Unio). (3) Cells of different origin have a different fate (numberless instances). (4) Cells of different origin have the same fate, *e.g.*, mesoblast in polyclads and annelids (*cf.* tables 5 and 7 in appendix), and the protoblasts of the prototroch and velum in annelids and molluscs respectively. Now what are we to say of such a series of contradictions?

Simply that it is impossible, in the present state of our knowledge, to explain them all. But this much may be said: The most striking feature is not the contradictions existing, but the wonderful agreements. The first and third of the facts enumerated state the rule; the second and fourth, the exceptions. The second fact, which is the first exception to the rule, may be explained satisfactorily, as I believe: The ovum, in any stage of development, is an organism complete in itself. Imagine that in any species a new organ is added, or rather, that a diffuse series of structures gains great importance and compactness in the course of evolution. Then, this new structure *may be* represented in ontogeny by a cell. But the form of cleavage is already defined, and each cell has its allotted destiny. The manufacture of a new cell being an impossibility, an old cell must be modified to represent the new organ. In other species, however, the same cell retains its original functions. This hypothetical case is similar, as I shall attempt to show, to the actual case cited above (*viz.*, $a^{2.2}$ in Nereis and Unio).

The cell ($a^{2.2}$) which in Unio supplies the larval mesoblast, is in Nereis the left "somatoblast." The larval mesoblast forms, as the name implies, only transitory organs, *viz.*, the isolated muscle-cells, which span the primary body cavity in various directions (V, part III), and the adductor muscle of the larva, which is nothing but a bunch of these myocytes,[1] having nothing to do with the adult adductors (Schmidt, No. 31; Braun, Nos. 6–9). The myocytes are widely spread larval organs in annelids and molluscs; in Unio they have increased greatly in number and importance, and have come to be represented in cleavage by a single cell, which thus must differ in fate from the cell of the same lineage in other forms.

If we can speak of an homology of cells of the same lineage in so many annelids and molluscs, it does not, of necessity, follow that the homology must be extended to other forms of the same type of cleavage. I agree with Wilson (No. 64, p. 455) that "the fundamental forms of cleavage are primarily due to mechanical conditions, and are only significant morphologically in so far as they have been secondarily remodeled by processes of precocious segregation." But the fact that the segregation has taken place in the same way in such widely separated instances as have been quoted above, is surely of the widest significance, whether we take it to mean that the ultimate fate of a cell is a function of its position in the cell-complex or not. *It is parallel precocious segregation in different cases that conditions cell homologies.*

Almost every detail of the cleavage[2] of the ovum of Unio can be shown to possess some differential significance. The first division is unequal. Why? Because the anlage of the immense shell-gland is found in one of the cells. The apical-pole cells divide very slowly and irregularly, lagging behind the other cells. Why? Because the formation of apical organs is delayed to a late stage of development. The second

[1] I propose this term "myocyte" for the unicellular muscles spanning the primary body cavity in so many larvae; and functioning as retractors or protractors of the velum or prototroch, *etc.* We have no convenient name in English for these cells, which are known to the Germans as "*Strangzellen.*"

[2] I do not include in this the oblique character of the cleavage or its general form.

generation of ectomeres is composed of very large cells. Why? Because they form early and voluminous organs (larval mantle). The left member of this generation is larger than the right. Why? Because it contains the larval mesoblast. The entomeres are very minute. Why, again? Because the intestine remains rudimentary until a late stage; thus a parallel instance to the apical-pole cells. One can thus go over every detail of the cleavage, and knowing the fate of the cells, can explain all the irregularities and peculiarities exhibited.

These peculiarities of cleavage are all due to the precocious segregation of organs or tissues in separate blastomeres. The order and character of the segregation again are ruled by the needs of the embryo. Thus, one of its greatest needs is the large and powerful shell with which it is provided. The necessity of such provision being made has caused the production of a large shell-gland, which has impressed itself on the segmentation stages as the largest of their blastomeres. I could illustrate the principle in each of the cases just enumerated, but will be satisfied with repeating the introductory sentence of this paragraph in a more special form: The peculiarities of the cleavage in Unio are but a reflection of the structure of the glochidium, the organization of which controls and moulds the nascent material.

The larval mesoblast of Unio must be regarded as a kind of massed mesenchyme, which has relations to the primary body cavity only. I consider it extremely probable that in other lamellibranchs, and in a wide series of forms besides, ectodermal cells pass into the primary body cavity and function as mesenchyme, producing muscle-cells, or, as in the case of the head kidney of Nereis, organs of excretion, or, again, supporting-tissue. Goronowitsch (No. 44*a*) and Miss Platt (No. 56*a*) have both recently certified to the derivation of the bases of certain tissues of the head of vertebrates, hitherto considered mesenchymal, from the epiblast. It may seem fanciful to collate such scattered observations from so widely different classes of animals, but I believe not. On the contrary, it seems to me that the very separateness of these observations gives them a significance all the more apparent. It will help

to convince embryologists that all tissues lying between ectoderm and entoderm are not, of necessity, either mesothelial or mesenchymal, in the Hertwigian sense, and that the occurrence of other elements is not isolated. It does not follow, therefore, that we must deny the homology of the mesoblast throughout. On the contrary, the tendency of the work being done, both on Vertebrata and Invertebrata, is to demonstrate that a portion, at least, of what was previously called mesoblast, is strictly homologous, both in origin and fate, within the limits of the Vertebrata and Invertebrata, respectively. When, for instance, we see that, in a widely varying series of Annelida and Mollusca, the mesoblast is derived from a cell of identical lineage, we must grant that a new and strong proof of homology is adduced. When, on the other hand, we meet with a form like Unio, where another conspicuous source of mesoblast is found, we shall decide on *a priori* grounds that such a source probably exists in other forms, but that it is comparatively inconspicuous.

Applying the test of observed facts to this decision but confirms its justice. Ziegler (No. 67), for instance, says, in speaking of the mesenchyme of Cyclas Cornea (p. 531), "Es ist mir daher nicht unwahrscheinlich, dass an bestimmten Stellen des Ectoderms Mesenchymzellen vom Ektoderm aus entstehen." Here is another lamellibranch in which it is "not improbable" that the ectoderm contributes to the mesoblast. What is the fate of these "fraglichen Zellen"? They form the single muscle-cells (Strangzellen) which are so numerous in the larvae of lamellibranchs. Now, as I shall show, the same cells in Unio are derived from the larval mesoblast, also the source of the larval adductor muscle, which has nothing whatever to do with the adductors of the adult (F. Schmidt, No. 31 ; Braun, Nos. 6 to 9); it is, in fact, nothing but a bunch of Strangzellen associated for a common end. I shall speak of this in more detail in the third part of my paper. Stauffacher, the last author on Cyclas, seems to regard ectodermal participation in the formation of the mesenchyme as very probable. Lankester (No. 54) is very positive about the derivation of many of the "branching cells" within the segmentation

cavity of Pilidium from the ectoblast. I must call attention to Goette's figures of Anodonta, in which scattered mesenchyme cells are shown in the place occupied by larval mesoblast in Unio, and which could hardly have come from the teloblasts of the mesoderm. Compare, also, the position of the dissociated mesenchyme cells in front of the archenteron in Cyclas with the position of the larval mesoblast in Unio (text, Fig. 7). Fol held that the ectoderm contributed to the mesoblast in pteropods, heteropods, and pulmonates. His observations may still be partly true even though Knipowitsch (No. 49) has seen mesoblast pole-cells in Clione, and Rabl in Planorbis. May there not, too, be some glint of truth in Sarasin's wholesale deduction of mesoblast from ectoderm in Bithymia tentaculata, in spite of the fact that Erlanger (No. 43) has seen the pole-cells of the mesoblast, and has traced them back to the posterior macromere? Wilson has traced back the head-kidneys of Nereis to two ectoblastic cells; Kleinenberg (No. 48) believed in ectomesoblast for Lopadorhynchus. In fact, it would be wearisome to review all the statements in support of the ectodermal origin of some mesenchymal cells, which one could cull from the literature. Unfortunately, most of the statements are qualified (*cf.*, *e.g.*, Ziegler's remark, *supra*), and doubt has been thrown on the rest. It may be, however, that the pendulum has now swung too far in the other direction. I believe in the complicity of the ectoderm in the formation of the mesenchyme. The coelenterate ancestors of the Mollusca possessed mesectoderm cells of contractile function. It would not be very strange if the undoubted phyletic continuity of ectoderm and mesoderm should repeat itself in ontogeny. (*Cf.* Kleinenberg, No. 48, p. 202, *etc.*)

As to the question of the relation of the embryonic axes to the first and second cleavage planes, it seems to me that too much emphasis has been laid on one point, *viz.*: on the relation which the entomeres bear to the embryonic axes. There is a certain justification for this, inasmuch as the entomeres are as a rule so much larger than the ectomeres. But the fact that the ectomeres are given off in different directions from the entomeres has not entered into account apparently. In Crepid-

ula, in Umbrella, and in Nereis the first cleavage plane is said to be transverse and the second parallel to the future median plane. Why? Because it is found, when the embryonic axes are determined, that the entomeres *B* and *C* are on the right side and *A* and *D* on the left side. But no account has been taken of the axial relations of the ectomeres. In Unio the decisive factor was with me the second generation of ectomeres, simply because they are the largest cells. As the four cells in question lie anterior, posterior right and left, I said (No. 21) that the future transverse and sagittal axes were inclined at an angle of 45° to both the first and second planes of cleavage. But in Nereis, Crepidula, and Umbrella the relation of the second generation of ectomeres is exactly the same. In Umbrella there is a fourth generation of micromeres, three of the members of which are entodermal and one (the posterior) mesoblastic. This fourth generation bears the same relation to the embryonic axes that the second does. The primary mesoblast *M* in Nereis, in Crepidula, and Unio as well, lies in the middle line behind. On the other hand, in Nereis, Crepidula, Umbrella, and Unio the first[1] and the third generation of micromeres have the same axial relations as the entomeres. Briefly expressed: the members of the odd generations of ectomeres, as well as the entomeres are distributed, two each, right and left of the middle line; those of the even generations are placed anterior, posterior, right and left. So that if the orientation be based on the odd generations or on the entomeres, it will be said that the first and second cleavage planes are transverse and longitudinal respectively to the embryonic axes; if based on the second or fourth generations of micromeres, it will be said that the first and second planes of cleavage are inclined at an angle of 45° to both transverse and longitudinal embryonic axes. Unio thus agrees completely in this respect with Nereis, Umbrella, and Crepidula.

There are forms, however, in which the relations are reversed.

[1] There is a wheel within a wheel here, inasmuch as the divisions of the first generation of micromeres, being oblique, cause certain of the resulting cells to lie on the longitudinal and transverse middle lines.

That is, in which the entomeres lie anterior, posterior, right and left respectively. If the oblique nature of the cleavage remains the same in these forms as in those above cited, the relation of the various generations of micromeres also to the embryonic axes must be different. The best studied forms which show these relations are Clepsine, Planorbis, and Neritina. In Clepsine (Whitman, Nos. 61 and 62), for instance d^2 (x^1 of Whitman) the neuro-nephroblast, or first somatoblast, does not lie in the middle line at first, but to the right (Whitman, Figs. 26, 33, and 34); for some time its products are asymmetrically arranged, but gradually become shifted into bilateral symmetry. In Clepsine *D* forms the mesoblast immediately after the budding off of d^2. There may be some correlation between these anomalies of cytogeny and the reversed relations of the blastomere generations to the embryonic axes. But at present we are unable to explain why, when widely separated forms agree, nearly related species should show reversed relations. The first careful study of the second condition will no doubt throw much light on this subject.

Studies of cell-lineage have an important bearing on the nature of differentiation. Any one who watches a segmenting ovum sees the differentiation of the parts which ultimately compose the adult organism going on under his eyes. It is too soon to say that mere observation of the phenomena accompanying differentiation will teach us nothing of its determining factors. As well say that the mere study of the facts of variation will teach us nothing as to the causes of variation! It is a hopeful sign that baseless hypotheses as to the nature of both these phenomena are giving way to a patient study of the accompanying facts.

A tremendous advance has been made since the time when it was thought sufficient to say, "by a series of rapidly succeeding cell-divisions the ovum is cut up into a great number of segmentation spheres, which arrange themselves in the form of a hollow ball." The first segmentation plane has since been shown often to have a definite relation to the future axes of the body; the various cells are not undifferentiated or equivalent, but destined for definite positions and functions in the larval

body. In other words, the blastomeres of segmentation stages have been shown to be the elements of a mosaic. This is a fact which no amount of argument or experiment can remove.

It remains for us to find out how these parts are made, how put together. Inasmuch as we have not as yet the entrance to the room of the raw materials in the workshop, we must study the products, the blastomeres, as they are being formed and after their formation. We must stop the process at each stage and fix it for the most careful and leisurely of examinations; when we have studied every stage of a division known to be differential, and have analyzed all the observable factors, the relation of the chromosomes in the two resulting cells and in each phase of division; of the asters; of the general cytoplasm; and have found out where the earliest indications of the cleavage manifest themselves; then, if we remember that we are observing but the finishing touches to the most elaborate and delicate of mechanisms, it may be that we can argue with some soundness on the philosophy of differentiation.

Conklin has cited some suggestive facts in this connection (No. 41, p. 33): in Crepidula however unequal the division of the cytoplasm may be, there is always an equal division of the asters and of the chromatin. "Yet in those very stages in which the nuclei and asters are equal in size, the lobing of the cytoplasm may show beyond doubt that the division of the cell-body is to be very unequal." However, before the cells have separated, the asters have become proportional in size to the surrounding cytoplasm; soon after the separation, the nuclei become proportional in size to the cell-body. In Unio I have observed that the resting nuclei become proportional in size to the cytoplasm, though in the different cells they contain originally the same amount of chromatin. Boveri (No. 37*a*) has observed that polar bodies in Ascaris megalocephala will act in the same way as the female pronucleus when brought under similar conditions. How instructive is his account of the continuity of the form of the chromosomes in the germ-cells alone in Ascaris, while in the somatic cells this form is lost from their origin! (No. 37.) Observations of this sort

point out the way for future investigation, and give us good cause for hope that, when we know more thoroughly the phenomena which accompany differentiation, we shall not be so much in the dark as to its determining factors.

For many years unequal cleavage has been explained as due to the arrangement of yolk within the dividing cell. So long as it was considered unnecessary to determine the prospective value of individual blastomeres, hardly any other explanation of unequal cell-division in ontogeny was possible. If the cleavage of an alecithal ovum was unequal from the start, *e.g.*, the lamellibranch ovum, a satisfactory explanation was ready to hand: it was due to the inherited effects of the lost food yolk. If unequal divisions occurred at any stage of development, the presence of yolk was sufficient explanation. Similarly yolk was held to retard cell-division by hampering the free action of the cytoplasm. It has even been held that the rapidity of cell-division is proportional to the concentration of the protoplasm. It is of course true in many instances, that unequal cleavage is due to the presence of yolk, but this is, nevertheless, only one phase of a more general law:— Unequal cleavage is conditioned by the constitution of the segmenting ovum, and always means the precocious localization of an organ or set of organs in the larger cell. This organ may be the entoderm, in which case it is usually accompanied by yolk; but the inequality of the first two cells in the annelids and molluscs is the earliest visible indication of another differentiation, the larger cell containing the two somatoblasts. The more precocious the differentiation of the organs of the somatoblasts, the greater the difference in the size of the cells (*cf.* Unio). The two cells may be equal in size when the organs in question are not precociously developed. The same principles suffice to explain unequal divisions throughout the cytogeny. This of course traces back unequal cleavage to protoplasmic structure and is in agreement with Watasé, who says (No. 60, p. 294): "The cause of unequal cleavage in the various cases which we have examined appears to me to be an internal one due to the peculiarities of the particular protoplasmic structure which composes the segment or segments."

What determines the direction of the spindle and hence of the cleavage? Here again we cannot get back of the organization of the cell. No mechanical explanation will suffice. Let us look for a moment at the cleavages of X: The first position of the spindle is on its left side; the second position on the right side (Figs. 25 and 27); the third in the middle line towards the apical pole (Figs. 33–35); the fourth in the middle line towards the vegetative pole (Fig. 39). (See accompanying diagram.) In none of these cases does the spindle occupy more than a fraction of the diameter of the blastomere in question. The nucleus has been wandering through the cytoplasm from one side to the other, from the front to the back, stopping at various stations, and giving off a cell at each one. Finally the nucleus stops in the centre of the cell and a perfectly bilateral spindle (the fifth) is formed (Fig. 44). Why does it stop there? Is it because its environment has changed? If so, the change is such as to elude the closest scrutiny. In fact the cell is a builder which lays one stone here, another there, each of which is placed with reference to future development.

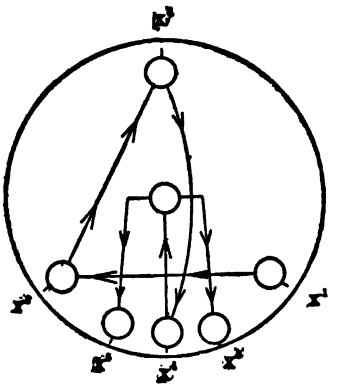


FIG. 2. — Diagram of the Cleavages of the First Somatoblast X.

III. GASTRULA TO GLOCHIDIUM.

(a) *Gastrula to Young Larva.*

1. ECTODERMAL ORGANS.

The formation of the shell-gland has already been described. Immediately afterwards the lumen is filled with a transparent, refringent substance, the first indication of the cuticular shell. A rapid evagination of the capacious gland now ensues. At the same time there is a thinning out, which proceeds more rapidly in the centre than at the sides. This is, of course, due to the extension of the area covered by the same cells. When

the evagination is completed the shell appears in the form of a very delicate, transparent, cap-shaped cuticle, covering the whole of the shell-gland area, and which is derived from the above-mentioned refringent secretion of the shell-gland.

As has often been noted, the shell is not at first bivalve; nor is it so when the gland has evaginated, and the dorsal line begins to undergo lateral compression (Figs. 68 to 71). The cuticular plate is merely bent over the dorsal surface, and adheres on each side to cells which represent the border of the evaginated gland, *and which are sharply marked off from their neighbors* (Pl. V, Figs. 75 and 76). The limits of the shell-gland are thus the limits of the shell. It will be noticed that the nuclei of the cells to which the margin of the shell is attached lie very near the surface, from which I conclude that they play an active part in the secretion and growth of the shell. It is not until some time later that the hinge makes its appearance.

The cells lying immediately beneath the shell become progressively thinner in proportion as the surface covered by the shell increases. Finally they become reduced to a mere film of protoplasm in which the nuclei produce swellings (Pl. V, Fig. 75; Pl. VI, Figs. 87 and 91, *etc.*). The growth of the larval shell takes place wholly from the cells which lie along its margin. As the cells which lie beneath the shell thin out, this region of the embryo becomes perfectly transparent (Pl. V, Fig. 79).

Great changes in the form of the embryo take place at this time. The general character of the changes in question can be seen by a comparison, in serial order, of the figures from 64 to 79. The most important elements of the change in form are two: On the one hand, the expansion of the shell-bearing region and the accompanying bilateral compression (*cf.* Figs. 62 and 68, with Figs. 76 and 80); and, on the other hand, the expansion of the anterior portion of the embryo; that is to say, the portion in front of the blastopore and of the shell-gland. (Compare this region in Figs. 66, 69, and 79.)

About midway between the blastopore and the cells of the head vesicle (*h.v.*) a slight depression becomes visible at the

time of invagination of the shell-gland (Fig. 69, *o.p.*). The bottom of the depression is occupied by numerous small cells very closely appressed, so that the cell-boundaries are seen only with difficulty. In whole embryos the region is marked by the cluster of active nuclei formed here. Flemming applied the name "Mittelschild" to this region in acknowledgment of his ignorance as to its fate; later he conjectured that it might form the oesophagus, but he was so uncertain about it that this can be regarded only as a lucky guess. Rabl at first took it to be the basis of the oesophagus: "Später aber überzeugte ich mich dass diese viel weiter oben am Vorderende des Körpers . . . entsteht und sich schon sehr frühzeitig mit den Entodermzellen verbindet" (Rabl, *l.c.*, p. 366). Schierholz was the first to clearly recognize its true significance. He applied to it the name "Mundschild," which I shall translate *oral plate.* The oral plate is destined to form the oesophagus, but it is a very long time before it does so and comes into function. Its place of formation is some distance in front of the anterior end of the blastopore, with which it cannot have had any connection at any previous stage. Later on, however, it moves bodily backwards and meets the ventral plate which at this stage marks the anterior border of the primitive mouth. (In another place I give the details of this process.)

This brings me to the consideration of *the closure of the blastopore and the extension of the ventral plate, two processes which go hand in hand.* In its early condition the blastopore has a considerable *antero-posterior* extent, and is quite wide (Pl. V, Figs. 67 and 68). Fig. 67 illustrates the important point that even at this early stage *the anterior and posterior lips of the blastopore are bounded by cells of very different appearance.* In later stages the distinction becomes much more evident (*cf.* Pl. V, Figs. 69 and 73). The posterior lip of the blastopore is formed by the small cells of the ventral plate (Pl. V, Fig. 67), which was elsewhere (p. 25) shown to be derived from the first somatoblast. The anterior lip is formed by the high columnar cells of the future larval mantle. The mesoderm teloblasts lie just behind the posterior lip. If Fig. 69 be compared with Fig. 67 it will be seen that, whereas the

same differences exist between the anterior and posterior lips, they are now near together. But the mesoderm teloblasts no longer lie immediately behind the posterior lip of the blastopore; they are separated from it by a wide space. It therefore follows that the blastopore has closed from behind forwards. How has this closure taken place? *By the forward growth of the ventral plate, which eventually meets the large columnar cells marking the position of the anterior blastoporic rim* (Pl. V, Figs. 69 and 73). Thus a plate of cells is established, extending from the hinder limit of the shell-gland to the anterior limit of the blastopore.

It is this plate of cells which becomes covered with cilia in embryos of Anodonta, the action of which causes a rotation of the embryo on its antero-posterior axis. In Unio such a rotation does not take place. It has, hence, been concluded by some (Rabl, No. 25) that the cilia are absent from this region. Others assert their presence (Schierholz, No. 30). With comparatively high powers of magnification (×600) I have been able to see very distinctly that this region is covered with extremely fine and active cilia in embryos of Unio. Small particles within the egg membrane which came in contact with the ventral plate were immediately swept away, and always toward the mouth region.

Considerable discussion has taken place as to the morphology of this ciliated tract. Thus it has been supposed to represent a rudimentary velum. The last idea published is, that they represent either the ventral or post-anal cilia of other lamellibranch embryos (Korschelt and Heider, No. 51). The question is of the morphology of the region bearing them. The region is that of the ventral plate, which, as I have already said, forms the foot and pedal structures, and, in addition, the post-anal region. The cilia in question, then, are homologous with those on the ventral surface of other Molluscan embryos. I shall have to postpone proof of this to a still later section.

A glance at Fig. 69 reveals the fact that just beneath the anterior end of the shell-gland there are large cells provided with large nuclei, each of which possesses a well-marked nucle-

olus. A surface section including these cells is shown in Fig. 71. They are six in number, the nuclei large and transparent, with usually a single very prominent nucleolus. Rabl has figured these cells. A comparison of his Fig. 29 with my Fig. 70 will leave no doubt as to their identity in the two cases. They have, of course, been seen by Schierholz, who has seen all that Rabl did. Rabl says of them (No. 25, p. 328): "An der dem späteren Hinterende entsprechenden Körperseite machen sich zu dieser Zeit drei durch ihre ausserordentliche Grösse und ihre kugelige Form von allen anderen Ectodermzellen auffallend abweichende Zellen bemerkbar"; and further on, p. 370: "nach meinen Beobachtungen entsteht diese Drüse" (thread-gland) "durch eine, zwischen drei, am Hinterende des Körpers gelegenen Zellen, auftretende Einstülpung des Ectoderms." He goes on to say that he cannot determine with certainty whether or not these three cells are derivatives of those mentioned before. It will be noticed that Rabl speaks of three cells, whereas I have figured six. As a matter of fact, three of these are more conspicuous in surface views than the other three.

According to my observations, Rabl is right in deriving the thread-gland from the region of these cells, but wrong in attributing its formation to invagination; *it is only one of these cells which forms the thread-gland proper*, and that is the one shown in the centre of the six (Pl. V, Fig. 71). Though smaller in superficial extent, this cell is really as large as those surrounding it, for it is much deeper. The six cells in question become more or less vacuolated; the nucleus of the central cell migrates to its inner end, the greater part of the protoplasm following it; finally, almost all of the cell lies within the primary body-cavity, but is still connected with the spot it formerly occupied by a strand of vacuolated protoplasm. The vacuoles run together, and form a lumen running from near the nucleus to the exterior. Almost immediately a refringent cuticular lining of the lumen is formed. The development of the gland may be traced through Figs. 73 and 74. The other five cells of the complex later surround the opening of the gland (Fig. 81; *cf.* Fig. 43 of Rabl). These

five cells persist in their characteristic position, and without any alteration of nuclear structure, until the glochidium is fully formed.

Fig. 74 is a part of Fig. 73 much more highly magnified. The nucleus occupies the inner end of the gland, and is identical in appearance with those between whose cells the gland opens. The lumen penetrates to within a short distance of the nucleus, and then disappears. Rows of granules may be seen radiating from the nucleus to the inner end of the lumen. The position of the gland, the character of its nucleus, and the presence of five nuclei only round its opening in place of the six original cells, make certain its derivation from the central cell of Fig. 71. The terminal nucleus persists for some time longer (Fig. 79), but gradually dwindles and disappears. During its existence it no doubt controls the growth in length of the gland, but after its disappearance the further growth seems to be supported by the five large cells around its opening.

Briefly, the further history of the thread-gland is as follows: It grows backwards beneath the hinge line until it reaches the posterior end of the body; then turns down, and passes on the right of the entodermic sac to the large cells of the larval mantle, which it enters at the angle of the shell. The apical nucleus has by this time disappeared. The gland continues its growth through the cells of the larval mantle, until it reaches its anterior end, when it turns dorsad to its opening beneath the anterior end of the hinge line. This is the extent of the gland in the stage represented in Fig. 82 (Pl. VI). In later stages a still greater length is attained. After the invagination of the mantle the gland takes two or three turns around the adductor muscle in the right valve of the shell.

How is the thread formed? It is hardly conceivable that such a long (10 to 15 mm. Forel) and strong structure should be formed by the activity of a single cell. Nor is it, in spite of the fact that the gland arises from a single cell. The thread must be thought of, not so much as a secretion into the lumen of the cell, as an actual metamorphosis of the substance of the cell (Pl. VI, Figs. 85, 91, *etc.*). When this has begun (in

the stage of Fig. 82) numerous mesoderm cells apply themselves to the gland and completely encase it. The further growth and secretion seems to be supported by these cells. The extrusion of the thread takes place some time before the rupture of the vitelline membrane.

One of the earliest and most natural ideas in regard to this organ was, that it was the homologue of the byssus-gland of other lamellibranchs. Rabl seems to have adopted this idea; he at any rate uses the term byssus-gland. Carrière (No. 10) was the first to show that this view is untenable, both from the position of the organ, and also from the fact that an actual byssus-gland is formed in the parasitic larva. He came to the only tenable conclusion, *viz.*, that it is an organ *sui generis*. As such it is still regarded, and I have found no characteristic which would cause it to rank with other organs elsewhere. But the morphology of the region which it occupies is very imperfectly understood; it is this latter question which I wish to clear up.

Ziegler (No. 67) suggested that the three bladder-like cells described by Rabl just in front of the shell-gland were a rudimentary head-vesicle. In the third volume of their text-book, Korschelt and Heider advance the same idea. This suggestion contains but part of the truth; they are, in fact, but part of a rudimentary head-vesicle. The head-vesicle in molluscan larvae is the region in front of the velum, which passes just in front of the shell-gland and of the mouth on the dorsal and ventral sides, respectively. The homologous region is thus easily defined in Unio. It is represented by the larger part of the region in front of *o.p.* (Pl. V, Figs. 69, 73, 79), and of the shell-gland or shell. It is thus seen to be quite an extensive area. The thread-gland opens at its dorsal limit in the middle line. It thus lies very nearly in the course which the velum would take if present, and I was therefore at first inclined to interpret its protoblast, with the similar cells surrounding it (Fig. 71), as cells of a rudimentary velum. However, comparison with Ziegler's figure (No. 67, Fig. 6) convinced me that the cells in question were really cells of a rudimentary head-vesicle. If Ziegler's Fig. 6 be compared with my Fig. 69,

a very striking agreement will be found in this respect. Just in front of the cells of the shell-gland in both cases are large rounded cells with large nuclei (text, Figs. 7 and 8 *h.v.*). In Cyclas these cells enter into the formation of the head-vesicle. In Unio, the head-vesicle not being developed, we can only regard these cells as a part rudiment of that once important structure.

The cell which forms the thread-gland is thus one of the cells of the head-vesicle. No such use is made of these cells elsewhere and so the organ must retain its rank as morphologically *sui generis*. But is it so physiologically? According to the generally received idea that the function of the thread is merely to assist the larva in attaching itself to its host, it is. It seems to me, however, that this cannot be its sole function. It certainly cannot have been the primitive function; for if it be of such assistance to the larva, it can only be in virtue of the length and strength of the thread secreted, and such an extensive structure could hardly come into existence fully formed. Its primitive function, both ontogenetically and phylogenetically, was probably excretion. Let us see what evidence there is in the actual development. I have already called attention to the rows of granules which radiate from the nucleus toward the lumen of the gland in early stages (Pl. V, Fig. 74). I take these to be an indication of active secretion on the part of the gland.[1] But after the terminal nucleus has disappeared, and this kind of excretion has ceased, matter still continues to be excreted through the gland, only now in the form of a solid substance, the thread. It is not necessary to assume that the function of excretion is lost simply because the products of excretion are utilized. Instances of utilization of waste products (so called) on the part of the animal producing them are by no means rare. This is moreover the only active larval organ which communicates freely with the exterior. If its

[1] It may, perhaps, be worth while to call attention to somewhat analogous function of cells of the prae-trochal region in Nereis. Wilson has discovered that two cells lying not far from the apical plate but behind it, move into the cavity of the head vesicle, where they acquire a lumen. He interprets them as head-kidneys; with a certain reservation be it said. These cells are paired, the thread-gland of Unio is, however, a median structure.

function be not that of excretion in the strict sense of the word, we would have the anomaly of an active larva without any provision for the excretion of waste products. The waste products in this case take the form of an insoluble substance as an adaptation to development within the strong vitelline membrane where soluble waste products could not but act injuriously. The thread-like form of the waste substance is the mechanical result of the form of the secreting gland. If the thread really assists the larva in attaining its host, this is a secondary and subordinate function.

Of the ectodermal structures of this stage it remains only to consider the rudiment of the larval mantle. This includes all the cells from the blastopore to the opening of the thread-gland. Its lateral extension is well shown in Fig. 79, all of the cells below the shell being included in it. It will be seen that the oral plate lies about in the middle of the area in question. The cells are cylindrical with the nuclei about the middle of their height. They resemble each other throughout the whole extent of the area, excepting in one narrow strip in the median line extending from the opening of the thread-gland to the oral plate (Fig. 80 *s.c.*). The cells in question were noticed by Flemming and called by him suture-cells ("Naht-zellen"). They are very narrow long cells and mark the line of division of the right and left halves of the mantle.

About this time (Figs. 79 and 80) appear the bristles which later become such a characteristic feature of the glochidium. There are but three pairs of these organs at this time (Figs. 79 and 80). Each is a little bunch (three to five in number) of stiff hairs born by a special cell. The position of these six cells is: one on each side of the thread-gland; one on each side of the oral plate, and one on each side of the anterior end of the ventral plate. A fourth pair is soon formed beneath the first pair mentioned.

2. MESOBLASTIC ORGANS.

We left the mesoblast in the stage in which eight cells are formed: four of the primary (*M*) and four of the larval mesoblast (*Y*) (Figs. 64, 65, 72, *etc.*). The cleavage of the parent

cells of the primary and larval mesoblast differs from the first. The divisions of the primary mesoblasts are teloblastic, those of the larval mesoblasts bear no such definite relation to the embryonic axis, but are irregular. The primary mesoblast cells adhere and act more like a mesothelium. The larval mesoblast cells are mesenchymal in their lack of coördination. This difference in the two kinds of mesoblast enables one to distinguish them throughout. In a considerably later stage (Pl. V, Fig. 69) the primary mesoblast forms definite germ-bands with terminal teloblasts. In front of the germ-bands lie the elements of the larval mesoblast.

At first sight there appears to be a considerable gap between the stage of Fig. 69 and the succeeding stage of Fig. 73, but the gap is more apparent than real, and is due to two changes. First the eversion of the shell-gland and secondly the appearance of the larval adductor muscle and myocytes. Even in the stage of Fig. 69 the adductor muscle and myocytes are foreshadowed; thus a myocyte is seen stretching from the oral plate to the entodermic sac.

The position of the mass of the larval mesoblast cells marks the position of the future adductor muscle. Moreover, and this is a fact of some importance, the nuclei of these cells resemble the nuclei of the early adductor muscle cells. Again, the primary mesoblast is a compact fundament in the earlier stage (Fig. 69); so is it in the latter stage. Here, however, transverse sections are necessary for its demonstration; two of these are shown in Figs. 77 and 78, taken in the planes marked by the lines (77) and (78) in Fig. 73. The most posterior section (78) shows the mesoderm teloblasts still plainly recognizable; they have, however, shifted their position to the sides of the entodermic sac (*cf.* Fig. 69). The primary mesoblast in front of the teloblasts is in the form of stout wings of cells stretching on each side from the entodermic sac to the walls of the body. In the place previously occupied by the elements of the larval mesoblast we have the larval adductor muscle and the myocites. When, in addition, the above mentioned similarity of nuclear structure is remembered, it is impossible to resist the conclusion that the larval adductor

muscle and the myocytes have been formed at the expense of the larval mesoblast.

The mere fact of the common origin of the adductor muscle and the myocites proves them to be formed from homologous elements. This fact, of course, includes their histological identity in early phases of development. As both F. Schmidt (No. 31) and Braun (Nos. 6–9) state definitely that no continuity between the adductor muscle of the larva and those of the adult exists, the conclusion that the larval adductor is merely an accumulation of myocytes is unavoidable. Thus another characteristic glochidium structure is brought into direct line with homologous parts elsewhere.

It seems almost superfluous to add that the distribution of the cells of the larval mesoblast throughout the primary body cavity does not convert the latter into a true coelom. This is, however, what Rabl has affirmed. Study of the post-larval development has shown that the true coelom appears much later, as the pericardium.

The larval muscle is composed at first of much elongated cells, with very granular cytoplasm and round nuclei (Figs. 75 and 76), each of which is provided with two distinct nucleoli. Later on the arrangement of the granules becomes very regular; the nuclei become oval with their long axis in the direction of the length of the cell; they are then drawn out into a rod-like shape and sometimes take up nearly half the length of the muscle fibre. This condition is reached in the stage of Fig. 91. This section, however, does not show the full length of any of the nuclei. In still later stages the muscle cells show a longitudinal fibrillation. Histological differentiation is then complete. I should add, however, that there is either a fragmentation or great shrinkage of the bacillus-like nuclei. For in the fully formed glochidium the muscle nuclei are quite minute.

Structures very similar in appearance to the "Strangzellen" are formed from the primary mesoblast; they are two stout wings of cells which run from the entodermic sac to the walls of the body, Not only are these different from the myocites in origin, but also in their fate. They soon fall into a clump

of small cells (Schmidt, No. 31 and Schierholz, No. 30), which are destined to form the pericardium, nephridia, and perhaps other mesoblastic structures. At no stage of their embryonic history are these cells contractile.

To return to the myocites. Schierholz has distinguished six on each side; but as of these, two pairs belong to the primary mesoblast, there are but four pairs of *myocytes* constant in position. The two most prominent pairs are shown in Figs. 75 and 76; the relative position of these will be better understood after reference to Fig. 73, in which the planes of the sections are indicated. Another pair passes from the oral plate to the entodermic sac (Fig. 69); and, sometimes at least, there is still another pair, partly indicated in Figs. 73 and 74, attached on the one hand just beneath the aperture of the thread-gland and on the other just in front of the oral plate. A very conspicuous strand is that shown in Fig. 79, running from the entodermic sac to the shell near the postero-dorsal border of the adductor muscle.

What is the function of these cells? Schierholz assigns to them an important mechanical part in development. Thus by their steady and slow contraction they produce invaginations, *e.g.*, the lateral pits, or the larval mantle; again they shift the relative position of the parts. These ideas are on a par with his suggestion that the oral plate is produced by the pressure of the overlying polar bodies (which as a matter of fact lie slightly in front of this plate). One cannot deny the originality of this view of the mechanics of development; neither can one accept it. The theory of unequal growth as the cause of invagination and kindred phenomena in development was never better illustrated than in the Unionidae. As a matter of fact, wherever in the embryo invaginations occur, clusters of active nuclei are to be observed (*cf.*, *e.g.*, the oral plate, Fig. 75, or the lateral pits, Fig. 95). It is true that myocytes are attached to the region of the oral plate; it is however quite common for the larval oesophagus to possess such attachments (*cf.* Hatschek, on Teredo). As for his theory of the lateral pits, the "Strangzellen" in that neighborhood are not myocytes, but derivatives of the primary mesoblasts.

The actual function of the myocytes is the same as in other animals: *i.e.*, to carry out temporary movements of parts. Some of them may be compared, though not perhaps homologized, with the retractors of the velum in Teredo for instance. The pair shown in Fig. 76 is doubtless that which functions later on in the movement of the hooks of the shell. Before the invagination of the larval mantle they all act as retractors of the soft parts.

3. ENTODERMAL ORGANS.

We left the entoderm in the form of a small sac communicating by a comparatively wide mouth, the blastopore, with exterior (Pl. V, Figs. 66, 68). In the next stage the blastopore is practically closed by the forward growth of the cells of the ventral plate area, and the entoderm is now represented by a small clump of cells lying in contact with the ectoderm. The dorsal and ventral lips of the blastopore meet, but do not fuse, so that it is possible to recognize the anterior end of the blastopore throughout. The entoderm generally assumes the form of a sac in the stage of Fig. 73 (*cf.* Fig. 77), but at other times no lumen is discernible. This is but an example of the usual variability of rudimentary structures.

4. SUMMARY.

In this section we have followed the development of the gastrula into the young larva. Before passing on to its transformation into the glochidium I shall briefly describe the young larva, comparing the names which I have used for the various parts with those already in use, to serve as a point of departure for the next section. In side view the young larva is roughly quadrangular; in transverse section, or end view, triangular (Figs. 79, 80). The straight hinge-line extends along the whole of the dorsal surface. Each valve of the shell covers about two-thirds of its side; the anterior, posterior, and ventral edges of the shell are curved (Fig. 79). The cells lining the shell are extremely flat; so much so, that the nuclei produce swellings. The ventral surface from the margin of the shell is formed of large columnar cells. About the centre of the

ventral surface is a shallow indentation, the floor of which is formed of small, closely appressed cells (oral plate; "Mittelschild," Flemming). Just beneath the anterior angle of the valves of the shell is the opening of the thread-gland, surrounded

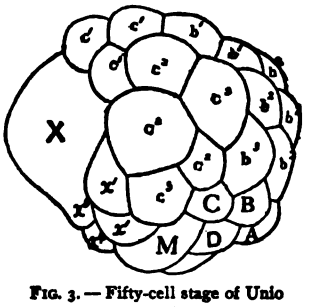


FIG. 3. — Fifty-cell stage of Unio from the right side.

FIG. 4. — Gastrula of Unio.

by five large cells (Figs. 80 and 81). The median line, from the opening of the thread-gland to the oral plate, is occupied by long cells drawn out in an antero-posterior direction. These are the suture-cells (Nahtzellen of Flemming), to which more attention will be directed in the next section. Below the posterior angle of the valves of the shell in the median line is a plate of ciliated cells; this I have called the ventral plate (Wimperschild of Flemming). These cells extend forward, and stop suddenly at the larval mantle cells (the columnar cells of the ventral surface already referred to). The place where the ventral plate meets these cells is the anterior limit of the blastopore. Lying within the ectoderm at this spot is the entodermic sac (Vorderwulst of Flemming), with mesodermic cells attached (Figs. 73, 77, and 78). The teloblasts of the mesoderm have now moved from their former position in the angle of shell-gland and ventral plate to just behind the entodermic sac. Two stout wings of cells pass from here to the lateral ectodermic walls (Pl. V, Fig. 77) (Seitenflügel of Flemming). Within the primary body cavity are numerous elongated

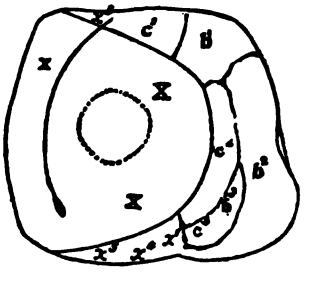


FIG. 5. — Young Larva of Unio.

mesodermic cells (myocytes; products of the larval mesoblast), some of which show a paired arrangement (Strangzellen of authors). Stretching across the primary body cavity from one shell valve to the other, nearer the anterior than the posterior end, is the larval adductor muscle, likewise a product of the larval mesoblast; dorsal to this, and running parallel to the hinge-line from the posterior end of the primary body cavity to open anteriorly between the five large cells already noticed, is the large unicellular thread-gland.

The accompanying text figures (3, 4, and 5) will make the relations of the areas more readily referable to the cleavage stages. It of course goes without saying that the areas in the second and third figures are only approximately correct in outline. The first figure is an actual reproduction of Fig. 47 (Pl. IV).

(b) *Transformation of the Young Larva into the Glochidium.*

Perhaps the simplest way of describing the transformation into the glochidium will be, first, to describe the glochidium, and then to ask how this form is derived from that of the young larva already described. Figs. 92 and 93 illustrate this description.

The glochidium larva of Anodonta possesses two triangular shell valves joined by their bases at the hinge-line. The valves are quite thick, strong, and brittle, and pierced by numerous fine pores. At the apex of each valve is a strong hook (provided with numerous teeth), which is quite different in appearance in Unio and Anodonta, being much stronger in the latter form, which I have figured. These hooks are joined to the valve proper by a hinge, and are moved by special muscles (myocytes). Each valve is somewhat spoon-shaped, and the cavity is lined by the larval mantle, consisting of large, flat vacuolated cells. The curve of the anterior edges of the valves is considerably greater than that of the posterior edges; the hinge-line straight, and of considerable extent. The valves are united by a strong internal ligament. The adductor muscle is very powerful, and, as in the young larva, is nearer the anterior than the posterior end. The larval mantle bears four paired

tufts of stiff, sensory hairs, arranged in a very characteristic manner. Three pairs lie just beneath, and within the powerful hooks; these three form the angles of a right-angled triangle, the base of which is parallel to the transverse plane of the larva, the apex being directed anteriorly. The fourth pair lie on either side of the opening of the thread-gland. These are, undoubtedly, the four tufts described for the young larva; but how different in their relative positions! As in the young larva, each tuft is borne by a single cell, the base of which is elongated in a peculiar manner, to be described more fully later on.

Near the posterior angle of the valves are two ectodermal pits—the lateral pits. Between them, beneath the ectoderm, is the entodermal sac. Behind this lie the lateral wings of mesoderm cells. The ventral plate occupies the whole of the posterior median region as far forward as the oral plate, which has now assumed the form of a stomadeal invagination. Just in front of the oral plate is the opening of the thread-gland, from which the long, much-tangled larval thread has been extruded.

The transformation of the young larva into the glochidium is attended by a series of shiftings and displacements of cells and groups of cells, which make this part of the development extremely difficult to follow. Flemming is the only author who has described these processes in detail; and indeed his description leaves little to desire in some ways. But Flemming was so uncertain as to the morphological meaning of the larval parts that he gave them all special names: "Wimperschild" = ventral plate; "Vorderwulst" = entodermic sac; "Mittelschild" = oral plate. It is due to this failure on the part of Flemming to recognize the homologies of these various parts that the apparent neglect of this part of his work is due.

The early appearance of the four paired tufts of hairs is of great assistance in following these changes. The arrangement of the sensory hairs on their first appearance has already been described. Their final arrangement may be seen in Fig. 93. To recapitulate: At first they are arranged in a row on each side, as follows: one tuft to the side of the thread-gland aperture, and a second tuft a little below this; the third lies just above the oral plate, and the fourth to the sides of the ento-

dermic sac.[1] During the metamorphosis the first two pairs move backwards with the thread-gland nearly to the oral plate. They thus come to be associated with the third pair of sensory hairs, which lie near the oral plate. When the invagination of the larval mantle takes place it is these three tufts which lie beneath the shell-hooks on each side. By this time the oral and ventral plates have grown together, and the fourth pair of sensory hairs now lies a little in front of the oral plate, and to the sides of the aperture of the thread-gland.

From the posterior angle of the valves of the shell to the oral plate—which is now assuming the character of a stomadeal invagination (in February glochidia of Anodonta)—the whole median surface is formed by cells of the ventral plate. The further growth forward of this plate is what causes the anterior displacement, in parasitic glochidia, of the oral plate to its definitive position. The foot—which is formed in this region—is then derived from cells of the ventral plate. It of course goes without saying that during these shiftings the larval mantle has invaginated. Having thus outlined the metamorphosis, it now remains to treat of each stage in detail.

The most striking difference between the young larva and the glochidium is the bifid condition of the mantle in the latter as contrasted with its unpaired condition in the former. It has been known since the time of Flemming's paper that the difference is established by the invagination towards the dorsal line of the whole ventral surface of the young larva along the median plane. But while this has in general been known, the histological changes which must accompany such a stupendous transformation have never been described. These are illustrated in Figs. 83 to 91. The preparation consists, first, in the establishment of a line of suture-cells (Figs. 80, 82, and 86) which divides the basis of the larval mantle into two halves, from the thread-gland to the oral plate; and, second, in the vacuolation of the cells which are to invaginate. Invagination

[1] This differs from Schierholz's description of their original position. It may be, however, that he overlooked their earliest appearance, when they are seen with difficulty. Schierholz described three pairs as lying near together, just beneath the edge of the shell, at the transverse level of the oral plate. This is not their original position in Unio.

commences, as might be expected, along the line of the suture-cells, and is at first most active near the thread-gland (Fig. 82). It is accompanied by very pronounced changes of form on the part of the invaginating cells. The greater mass of the protoplasm migrates to the inner end of the cells (Fig. 85); the nucleus accompanies it — a very usual appearance in large invaginating cells; the cells then roll up and in towards the shell, which thus comes to be lined by two layers on each side (Figs. 87 and 90): first, the protoplasmic layer already spoken of (which is very intimately attached to the shell); and, second, the cells of the invaginated mantle. Beginning, as I have said, in front, the invagination passes backwards, in proportion as the oral plate travels towards the ventral plate, which, on its part, moves forward to meet the former. *The rest of the mantle rudiment is thus divided into lateral halves and invaginates under the same appearances, carrying with it the median ventral and oral plates, which have now met and occupy the whole of the posterior region.*

The vacuolation of the larval mantle cells begins in quite an early stage—about the stage of Fig. 79. Flemming has figured and described the appearance of these cells at this time. The vacuolation, which is not at first very marked, soon becomes more and more exaggerated (Fig. 85). Indeed, it seems as though there was an active effort on the part of the cells in question, with a given amount of protoplasm, to cover the greatest space possible. The transition from the compact columnar cells of Figs. 76, 77, and 78, to the flat, much-vacuolated cells of Figs. 84 and 93 is most striking.

The suture-cells are well seen in Fig. 90 and in section in Figs. 85, 86, and 87. They are long, spindle-shaped, deeply staining cells, with rod-like nuclei; in cross section they are wedge-shaped (Pl. VI, Fig. 85).

During the invagination of the mantle, the thread-gland has shifted its position backwards along the line of the suture-cells, and now lies just in front of the oral plate. Although the displacement of the thread-gland takes place along the line of the suture-cells, yet I should hesitate to attribute any active share to these cells. They seem rather to be the preformed

path of displacement, and serve to separate the halves of the larval mantle as well. Schierholz has figured a muscle-cell connecting the thread-gland with the oral plate, and attributes to it the function of causing this displacement.

The ventral plate goes through some interesting changes (to which I have already referred) about this time. Figs. 83 to 89, representing sections in various planes through the stage of Fig. 82, illustrate the description. The ventral plate grows past the posterior margin of the larval mantle (which represents the anterior end of the blastopore) and towards the oral plate (Figs. 83 and 84), which is at the same time moving backwards through the larval mantle. At this time the oval plate can be recognized only as a cluster of deeply staining nuclei (Fig. 84), all traces of its previous pit-like condition being obliterated by the great expansion of the cells of the larval mantle. A tongue of cells of somewhat similar appearance to the ventral plate at the opposite end of the embryo might lead to the belief that similar processes of development were responsible for the two structures; and hence that the appearance of free, forward growth of the ventral plate was illusory. But that this is not the case is proved by horizontal sections (Figs. 86 and 87). These show that at the anterior end of the embryo (*i.e.*, directly opposite the ventral plate) there is a protrusion of the cells of the larval mantle without the shell, caused of course by the violent contraction of the adductor muscle on the addition of killing reagents. There is nothing of this sort to be seen at the posterior end. It is the section of the protruding cells which is seen in sagittal section at the anterior end. (*Cf.* lines of section in Figs. 84 and 87.) I have hence been forced to conclude that there is an actual growth forward of the ventral plate above the cells of the larval mantle. The oral plate has been moving backwards through the larval mantle at the same time. During the invagination of the mantle the two structures meet and unite (Fig. 88).

The mantle cells have rolled away to the side; and hence the whole of the median portion of the glochidium, from the oral plate to the posterior angle of the shell, is formed from the ventral plate (Fig. 93). According to the united testimony of

those who have studied the post-embryonic development, the anterior part of this area is the basis for the formation of the foot and pedal structures.

Schierholz derives the rudiment of the foot from the median portion of the cells of the larval mantle lying between the oral plate and the anterior end of the ventral plate. That I am unable to agree with him goes without saying.

It is practically certain that the anus forms behind the anterior limit of the blastopore, but still within the limits of the area originally occupied by its posterior portion.

It will suffice to merely mention the lateral pits (*cf.* Figs. 89 and 93 to 97) lying at the sides of the foot-fold, as we may now call the area of the ventral plate. They are covered with active cilia, which are in direct continuity with the cilia of the foot-fold (Fig. 93). The structure of the walls is shown in the sections (Figs. 94 to 97). Schierholz and Schmidt derive the gill-filaments from the outer walls of these pits. Within the pits, according to Schierholz, lie two or more rounded cells which he regards as the basis of the otocysts. I have sometimes seen such cells in the stage of Fig. 79 lying on the surface near the anterior end of the ventral plate, but it seemed to me that they did not persist. In any case it is difficult to see how they could represent the otocysts.

The cerebral ganglia have begun to form in some glochidia of Anodonta in February. A section through their rudiment is shown in Fig. 94, which is taken about 22 μ. in front of the stomodaeum.

The bristles which lie on each side of the thread-gland and beneath the hooks of the shell have been considered sensory by all authors who have mentioned them. I found that when the glochidia were left for some time in a weak solution of methylene blue the cells bearing these bristles were the only ones in the embryo which took the stain. After fixing with picrate of ammonia, long, stained protoplasmic processes of these cells could be traced for some distance beneath the larval mantle. In Fig. 86 I have shown the course of the processes of the lateral bristle-bearing cells. In no case was I able to make out any coördinating structure with which the processes

were connected, though one can hardly doubt that such a structure exists. Flemming and Rabl have figured somewhat similar but shorter protoplasmic processes to the bases of these cells. Their reaction to methylene blue seems to me fresh evidence of their sensory nature.

I do not include a detailed description of the cells in question; for that has already been done by Flemming and Rabl. I shall merely call attention to the conical form of these cells and their elevation above the surrounding surface (Fig. 92). They are supposed to transmit to the adductor muscle the stimulus which causes its contraction. The necessary stimulus might of course come from contact with the prospective host, in which case the contraction of the muscle would force the hooks into its skin, thus securing the requisite attachment (Schierholz).

Mesoblast.

In the stage of Fig. 82 and presumably somewhat later, the teloblasts of the primary mesoblast are still recognizable. The remainder of the primary mesoblast has fallen into a clump of small cells, which take up a position behind the entodermic sac as the latter moves forwards (Fig. 84). In the glochidium the mesoblast is very distinctly paired and lies in contact with the posterior walls of the lateral pits (Figs. 89, 94, and 97), stretching to the posterior end of the embryo on each side. A special wing of the mesoblast may be seen on each side behind the lateral pits. In well stained specimens this portion of the mesoblast shows but few clear nuclei with distinct nucleoli (Fig. 93). According to Schmidt, these cells are the fundament of the organ of Bojanus (the nephridia).

Some of the myocytes are specially modified as retractors of the hooks (Schmidt). The others are attached to the larval mantle and shell, and serve to keep the former in varying degrees of approximation to the latter.

Entoderm.

In embryos of Unio of the stage of Fig. 82 the entoderm no longer forms a sac, but has become a mass of cells (Figs. 83

and 84). It is already beginning to stretch forward towards the oral plate above the cells of the larval mantle. It is no doubt the mechanical cause of the splitting of the larval mantle, which permits the ventral plate to come in contact dorsally with the entoderm sac and to fuse anteriorly with the oral plate. In the glochidia of Unio, which do not as such reach so advanced a stage of development as those of Anodonta, the entoderm remains in this state till the post-embryonic development begins (*cf.* Fig. 89). In those glochidia of Anodonta, however, which have wintered in the maternal gills, the entoderm has already begun its differentiation. It has taken on the form of a sac which in one case ran through seven sections of 7½ mm. each (Figs. 95–97). Lateral expansions in the middle of its course I took for the liver diverticula from comparisons with Schmidt's sections of parasitic larvae (Fig. 96 *l.c.*). Anteriorly it was connected with the stomodaeum (Fig. 95) and posteriorly the end-gut was indicated (Fig. 97).

General Remarks.

One cannot view such a remarkable and unusual series of phenomena as accompanies the transformation of the young larva into the glochidium without asking one's self what is the reason of it all? Why, for instance, should the thread-gland be formed so far from its definitive position? The most natural explanation is that the primitive function of the organ in question has changed, and that a new position seemed more favorable for the discharge of the new function. On such an hypothesis there is nothing wonderful in such phylogenetic changes of position being repeated in ontogeny. We can easily apply this to the explanation of the displacements of the thread-gland. In its definitive position practically in the centre of the ventral surface, it manifestly occupies the best position for the discharge of its present function, which, as we have seen, is probably to assist the glochidium in attaining parasitic attachment to its host. For if the thread becomes attached, for instance to a fish's fin, the larva is pulled on to the fin ventral surface down; when the muscle contracts the

hooks are forced into the tissue of the fin. Now were the original position of the thread-gland to be retained, *i.e.*, at the anterior angle of the valves of the shell, the larva would not be likely to "land" on the fin in so favorable a position for attaining a secure footing. We have seen before that the primitive function of this gland was probably excretion; the change in function, then, has brought about a corresponding change in position.

I should not attempt to apply such an explanation to the movements of the oral plate and ventral plate, but would rather explain them as caused by the necessities of precocious segregation which must often isolate organs which later are intimately related.

Two factors are responsible for the redistribution of the sensory hairs in the glochidium, *viz.*, the backward motion of the thread-gland, and the invagination of the larval mantle. I have just considered the movement of the thread-gland; now as to the larval mantle. I call this the larval mantle because all authors who have described its later history state that it does not form (or does in part only) the mantle of the adult, but degenerates, giving us the so-called "fungus-like bodies" of Braun. The larval mantle is established certainly in a very curious way, and yet I think that a little consideration will convince us that at bottom it is not much different from the mantle of other forms. Its borders are formed by the evaginated edges of the shell-gland to which the shell remains attached. Practically the same thing is true for the embryonic mantle of all Molluscan forms. The difference is simply that the cavity is so enormous in this larva; and instead of being a groove-like cavity above the foot, deeper at the (primitive) posterior end, here the cavity is so great that the embryo lies within it at one end. Schmidt has attributed this concentration of the embryonic area to the immense development of the adductor muscle. It seems to me, however, an unnecessary assumption to make, for the parts in question no doubt occupy all the space they require. We should expect the embryonic material to assume a compact form, and its position at the posterior end is the natural one. The posterior end is always the growing zone of the embryo.

But why these parts are so small is a different question. We can recognize in this the degrading influence of parasitism. It would, indeed, be strange if this mode of life, which can so profoundly influence animals which have become adapted to it, as to render it a matter of speculation in what corner of the animal kingdom to classify them, had not left a deep imprint on the organization of this larva. The effect of parasitism is to exaggerate all organs essential, and to eliminate all that are inessential, to the parasite. This is precisely what has taken place in the glochidium. The larval thread, the strong muscle, the heavy shell with its hooks, and the larval mantle, are all essential to its peculiar mode of parasitism. The foot, the mouth, the intestine, the heart, *etc.*, *etc.*, are all inessential. The former have thus been enormously exaggerated, becoming precociously impressed on the cleavage of the ovum ; the latter have been reduced to mere rudiments, the reduction also leaving its imprint on the segmentation ; but they have not been eliminated, because they are functional organs in the adult.

This is one of the most interesting of all cases of parasitism, because we have an animal fully equipped as a larva for parasitic existence, and later leading an independent life. It shows us how far parasitism can go without eliminating the possibilities of a higher evolution. It seems strange that the parasitism should finish with the larval life ; but that it does so, and that, despite its short duration, the preparation therefor should so profoundly alter the characters of the larva, is one of the best examples of the oft-emphasized fact that natural selection deals no less with the larva than with the adult. We might search the animal kingdom through without finding a better example. What a bountiful supply of transitory organs of offense and defense has nature supplied to this larva ! and all that a passing and purely larval condition should be ensured in the greatest possible number of cases !

Another important question which suggests itself, is : Why has this brief parasitic period been intercalated in the life history of this animal ? I think that Schierholz answered the question very satisfactorily when he said that it was to avoid the injurious action of the fresh water on the delicate shell of

the young animal. As parasitism gradually became the fixed habit of the species, the adaptation to the requisite conditions became more and more perfect, until the parasitism became a necessary consequence of the structure, and an indispensable condition of development.

The parasitism of the glochidium is but one way of securing protection against the injurious effects of fresh water on delicate larvae. The same protection is assured other forms (turbellarians, Cyclas, gasteropods) by a foetal development, which takes place either in the body of the mother or in impervious capsules. The mode of securing this end among the Unionidae is evidently correlated with the enormous number of young produced. Not that the enormous number of the young made actual viviparous reproduction such, *e.g.*, as in the Cycladidae, impossible, and hence forced, so to speak, the species to devise another means of protection. It is more logical to hold either that the parasitic habit preceded the production of such a multitude of larvae, or else that both evolved *pari passu*. We cannot, at any rate, suppose that a species could be thus perpetuated if only a few young were produced, so precarious and uncertain is the attainment of the necessary conditions of higher development.

If we suppose the Unionidae to have been derived ultimately from a marine form, we are offered a hint as to the possible mode of evolution by the present condition of Dreissena, which is evidently undergoing a similar change of habitat. The development of this form is a metamorphosis with a free-swimming larva, which hardly differs from the marine larvae of the same class. It is easy to picture two or three possible courses of evolution open if it becomes completely adapted to life in fresh water (in which case, as all our experience tells us, the larva would be lost). In the one case it might become purely viviparous like Cyclas, and produce but few young; or the ova might be deposited in impervious capsules (as, *e.g.*, pulmonates); or, again, the larva might in some way become adapted to parasitism, with consequent protection to its delicate structure. It is practically certain that in this last event the larva would change in two ways: First, so as to make it an

efficient parasite; and second, so as to be protected, as far as possible, during its brief but necessary contact with the fresh water. To ensure the continuance of the species an enormous number of young would have to be produced. With the aid of some such an hypothesis the curious ontogeny of the Unionidae becomes more comprehensible.

(c) *Axial Relations.*

I have left till the last the consideration of the trochophore stage, and the axial relationships of the larva in the Unionidae, because it seemed better to have the whole course of develop-

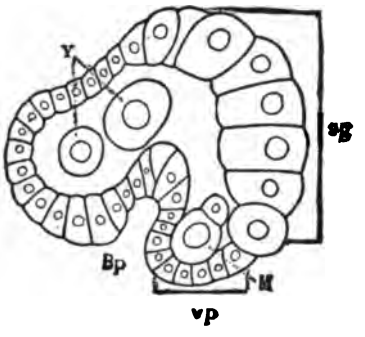


FIG. 6. — Gastrula of Unio.

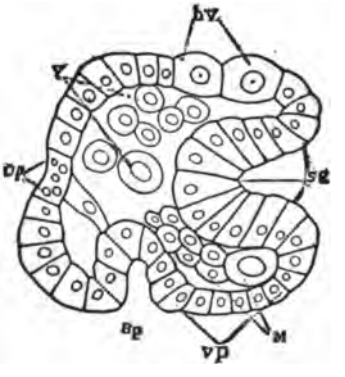


FIG. 7. — Slightly older Gastrula of Unio.

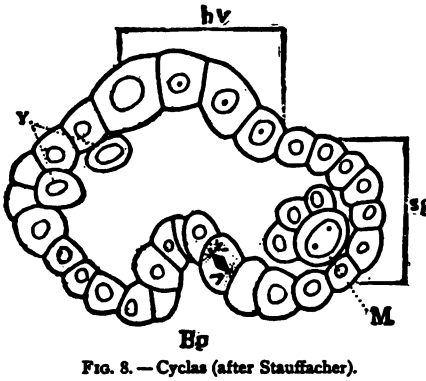


FIG. 8. — Cyclas (after Stauffacher).

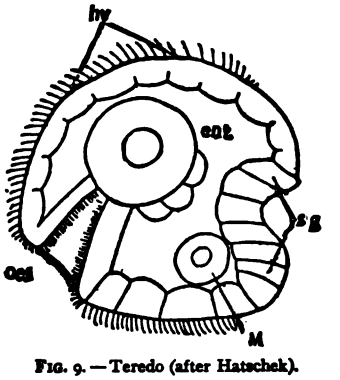


FIG. 9. — Teredo (after Hatschek).

Bp. Blastopore.
h.v. Head-vesicle.
o.p. Oral plate.
v.p. Ventral plate.
Ent. Entoderm.
M. Mesoblast.
oes. Oesophagus.
s.g. Shell-gland.
Y. Larval Mesoblast.

ment in mind in such considerations. The accompanying text figures (6 to 9) require but little explanation; they show that there is no real difficulty in recognizing the homologous areas in Unio, Cyclas, and Teredo. The latter is one of the most typical of the marine veligers; that is to say, approaches most nearly the trochophore in its structure.

Of the typical trochophore organs, the apical plate with its tuft of cilia, the praeoral and postoral rows of cilia and the head kidney are missing in Unio. These are of course among the most characteristic organs of the trochophore and most essential to the free life of the larva. It is these organs which always degenerate more or less subsequent to the giving up of the

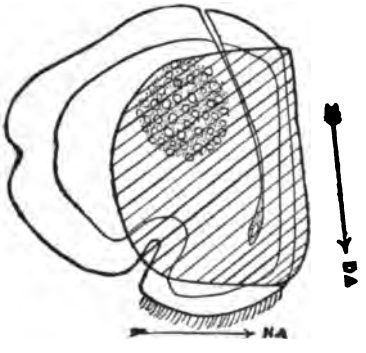


FIG. 10. — Young Larva of Unio.
N.A. Neural Axis. D.A. Dorsal Axis.

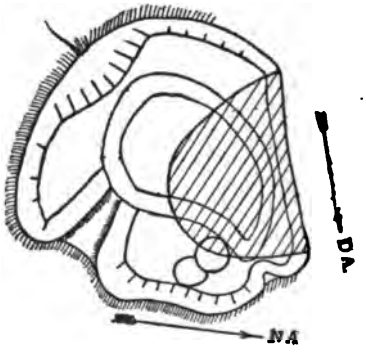


FIG. 11. — Teredo (after Hatschek).
N.A. Neural Axis. D.A. Dorsal Axis.

free life. A complete series can be traced through the various degrees of degeneracy of the organs in question to their complete absence in the Unionidae, where we can recognize only the homologous areas. The swollen cells of the head vesicle are the only remaining differentiation of the apical area which can be interpreted as rudimentary trochophore organs.

It is important to notice that in the veliger stage of all Mollusca the long axes of the shell or shell-gland and of the foot, which in the adult are parallel, are inclined at an angle of nearly 90° to one another. The figures 10, 11, and 12 in the text illustrate this in Anodonta, Ostrea, and Teredo. Even the most cursory examination of Gasteropod larvae will show that the same thing occurs there. This is due to the fact that the dorsal and ventral surfaces of the trunk are independently

established in these forms. The two most important factors in establishing the adult relations are the growth of the shell-gland, *i.e.*, dorsal region, and of the foot respectively. The shell-gland assumes the adult relations first owing to its early importance; the foot or neural axis is established later; this is in adaptation to its lack of function in the trochophore.

These axial shiftings have often been referred to. It will, nevertheless, be useful to review shortly the clearest accounts of them. Fol (No. 44) gives a remarkably straightforward account of the axial shiftings in the pteropods and heteropods. His statement in his pteropod paper is so concise

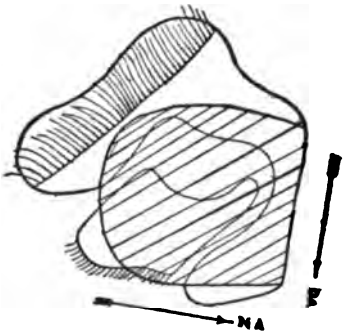


FIG. 12. — Ostrea (after Horst, from Korschelt and Heider).
N.A. Neural Axis. D.A. Dorsal Axis.

that it may be quoted entire (l.c., p. 202). "En effet, aussitôt que les deux feuillets primitifs de l'embryon sont formés, le feuillet externe se met à croître et à s'étaler beaucoup plus rapidement d'un côté que de l'autre. Ce côté à croissance rapide répond à la région ventrale et postérieure de la larve, à celle où se trouve, on se le rappelle, la sphérule primitive protoplasmique, à celle qui donne naissance au pied et au manteau. Le tissu ectodermique qui occupait dans l'origine le pôle formatif subit de la sorte un déplacement relatif et paraît remonter le long du dos de l'embryon pour arriver enfin à la région céphalique."

"La sphérule primitive protoplasmique" to which Fol refers is the posterior macromere. The mantle and the foot, the whole trunk in fact is traced back to the posterior macromere.

In his heteropod work Fol is, if possible, even more explicit. He shows that the region of the shell-gland is at first posterior, and that gradually it comes to lie above the mouth, *i.e.*, dorsally; at the same time the upper pole, as marked by the polar globules, is pushed around anteriorly until finally it lies at the anterior end of the body. The foot area has expanded at the same time. Thus there is a change of axis which has been accompanied by the formation of new regions, viz.: the shell-gland (dorsal surface), and the foot (ventral surface). Where these two regions meet posteriorly there must be a stationary area, a zone of growth. This region of growth corresponds in position to the first somatoblast of Unio.

Later authors describe a stationary area in the region indicated. Conklin (No. 40) says, "The cells of the posterior arm (of the cross) enlarge greatly and are carried forward until they lie over or even anterior to the cross-furrow, while the point at which the polar bodies are attached (the centre of the cross) is carried forward through an angle of about 90° so that it finally lies at the anterior end of the long axis of the embryo. The position which the polar bodies first occupied (immediately over the cross-furrow) coincides with the middle of the dorsal area, while the ectoderm cells which immediately surround the ectoderm pole are carried forward until they lie at the cephalic pole of the embryo. The endoderm seems to take no part in this shifting, and the ectoderm on the posterior side of the ovum is not shifted forward, but grows around in the opposite direction. There is thus a stationary point in the ectoderm on the posterior side of the ovum in front of which the ectoderm cells are shoved forward, and back of which they are shoved backward and downward. This stationary point coincides very nearly with what is later the region of the shell-gland."

This stationary point coincides also very nearly with the region of the first somatoblast. It must be a region of proliferation, anteriorly and posteriorly.

Heymons (No. 47) has witnessed the same phenomena in Umbrella. He says, p. 26 : "Hieran sind lebhafte Wucherungsprocesse im Ektoderm betheiligt. Dieselben schliessen sich im wesentlichen an die neuerdings auch von Conklin be-

schriebenen Erscheinungen an. Im hinteren Theil des Ektodermfeldes beginnen sich die Zellen mehrfach zu theilen, und nach vorn fortzuschieben, während gleichzeitig das durch die Richtungskörper gekennzeichnete Centrum des animalen Poles allmählich an das Vorderende gelangt. Nur der hinterste Theil des Ektodermfeldes nimmt an dieser Verschiebung keinen Antheil, sondern wuchert weiter nach hinten, d. h., nach dem vegetativen Pol hin. Die beiden Urmesodermzellen wurden dadurch gewissermassen vom Ektoderm entblösst, oder doch nur von sehr wenigen plattenförmig ausgebreiteten Ektodermzellen an ihrer dorsalen Fläche bedeckt. Letzere stellen damit die Grenze zwischen der nach vorn und der nach hinten wachsenden Partie des Ektoderms dar. Unmittelbar vor ihnen macht sich später, wenn die geschilderten Vorgänge beendet sind, weiter eine starke Vermehrung und Anhäufung von Ektodermzellen bemerkbar, die sich später in das Innere einsenken, und die Anlage der Schalendrüse bilden."

If this region of proliferation were traced further back it would probably be found that it was referable to a single cell, viz.: the first somatoblast. This is what I have done in the case of Unio. The whole ectodermal trunk region is thus traceable to the first somatoblast, the second product of the posterior macromere. The mesodermal elements are traceable to the same macromere. The whole trunk region behind the mouth can thus be traced back step by step to the posterior macromere. Dr. Whitman showed that this was true of Clepsine as far back as 1878, and Wilson has shown essentially the same thing for Nereis.

It seems to me that these facts afford a new basis for comparison of the trunk of Annelida and the postoral shell- and foot-bearing region of Mollusca. They correspond in position and in their relation to the germinal layers ; it seems also that they can be traced back to identical blastomeres. I must confess that Unio is a form but little adapted to place this question beyond dispute. I have, however, the utmost confidence that in less highly modified forms this position will be sustained.

UNIVERSITY OF CHICAGO,
May, 1894.

APPENDIX.

The appendix includes the results of some of the most important works on the cell lineage of worms and molluscs reduced to tabular form. It is inserted as a possible convenience to other workers in the same field. The system of naming the cells is the one employed in this paper, with the original designations in brackets. The necessary remarks have been made as brief as possible.

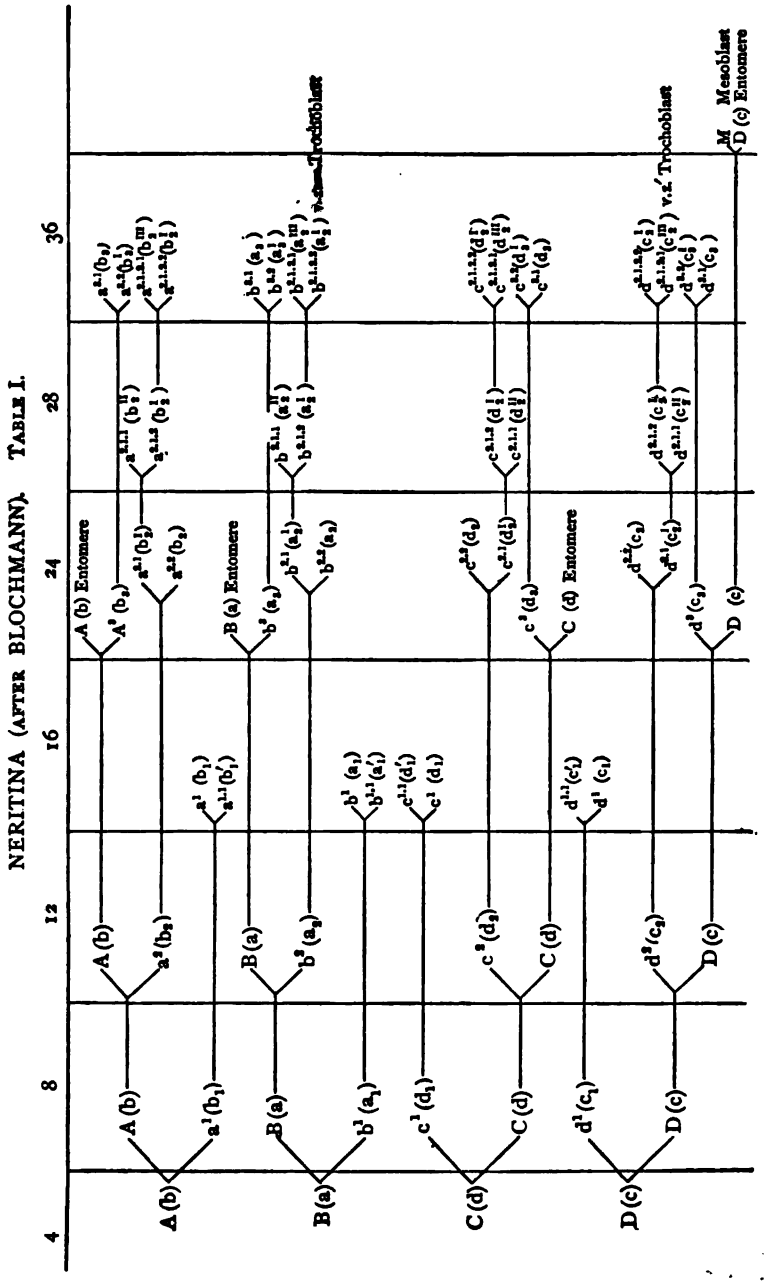
Kofoid has already published a criticism of Blochmann's work (Table I) based on the internal evidence, and I can only concur in his judgment. Referring for the evidence to Kofoid's preliminary paper (No. 49[e]), I will merely note the probable errors. The trochoblasts are, according to Blochmann's derivation, $d^{2.1.2.1}$ and $b^{2.1.2.1}$ (*v.* table); but there is almost no doubt that the cells in question are a^3 and c^3. The inner cell of the cross would be $a^{2.1.1}$ to $d^{2.1.1}$ according to Blochmann's derivation, whereas there is but little doubt that it is $a^{1.2}$ to $d^{1.2}$, thus a member of the first group of micromeres. It is, however, rather unsatisfactory to criticise a work from internal evidence alone; but until such manifest discrepancies between text and plates, as occur in Blochmann's work, are explained, it is impossible to place great reliance on them.

Rabl (Table II) has evidently been in error in the orientation of the embryos. If we are to accept his figures, the first generation of micromeres is formed leiotropically. The second generation is formed in the same way. It rotates the first generation of micromeres still further to the left. The rotation goes on, according to the figures, until finally a^1 is above C and c^1 above A. That is to say, what was on the right side is now on the left side of the embryo and *vice versa;* similarly what was anterior (of the first generation of micromeres) has become posterior and *vice versa.* It seems difficult to accept this as being really true. The first error, it seems to me, was probably in his assigning the members of the first generation of micromeres to wrong macromeres as parent cells. Thus E should not have been assigned to EJ, but to ME, *etc.*, which would make the direction of its formation dexiotropic.

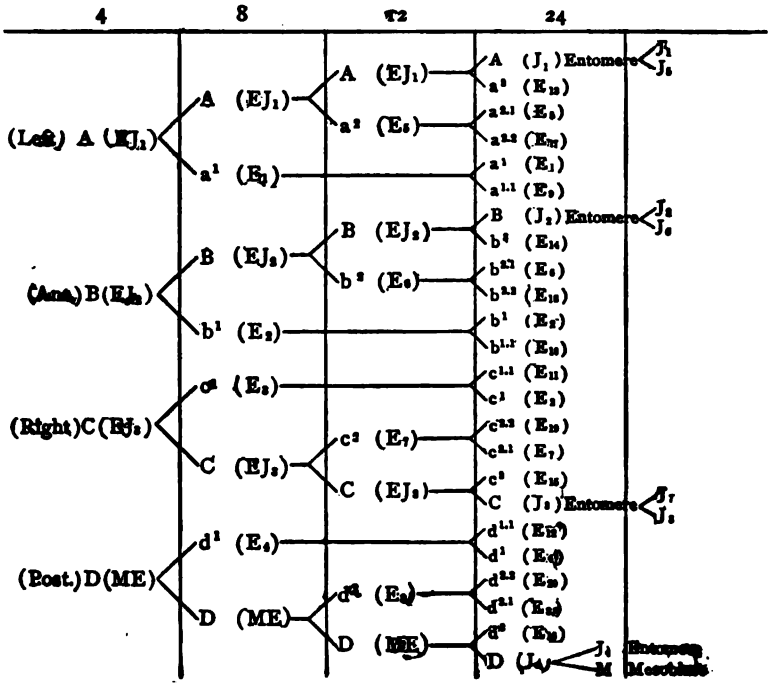
It seems to me further probable that the orientation of his figure 12*a* is incorrect; it will be noticed that the cross-furrow between E_2 and E_4 is at right angles to its direction in 11*A*. It is easier to believe that Rabl has mistaken one side of the embryo for the anterior or posterior end. This might easily be done; for the macromeres are equal in size and the only means of orienting them is the cross-furrow, which is invisible from the apical pole. Thus the apical cross-furrow in 12*A* is probably at right angles to the vegetative as in 11*A*, and not parallel as represented. This table also shows fairly accurately the cleavage of Limax according to Kofoid.

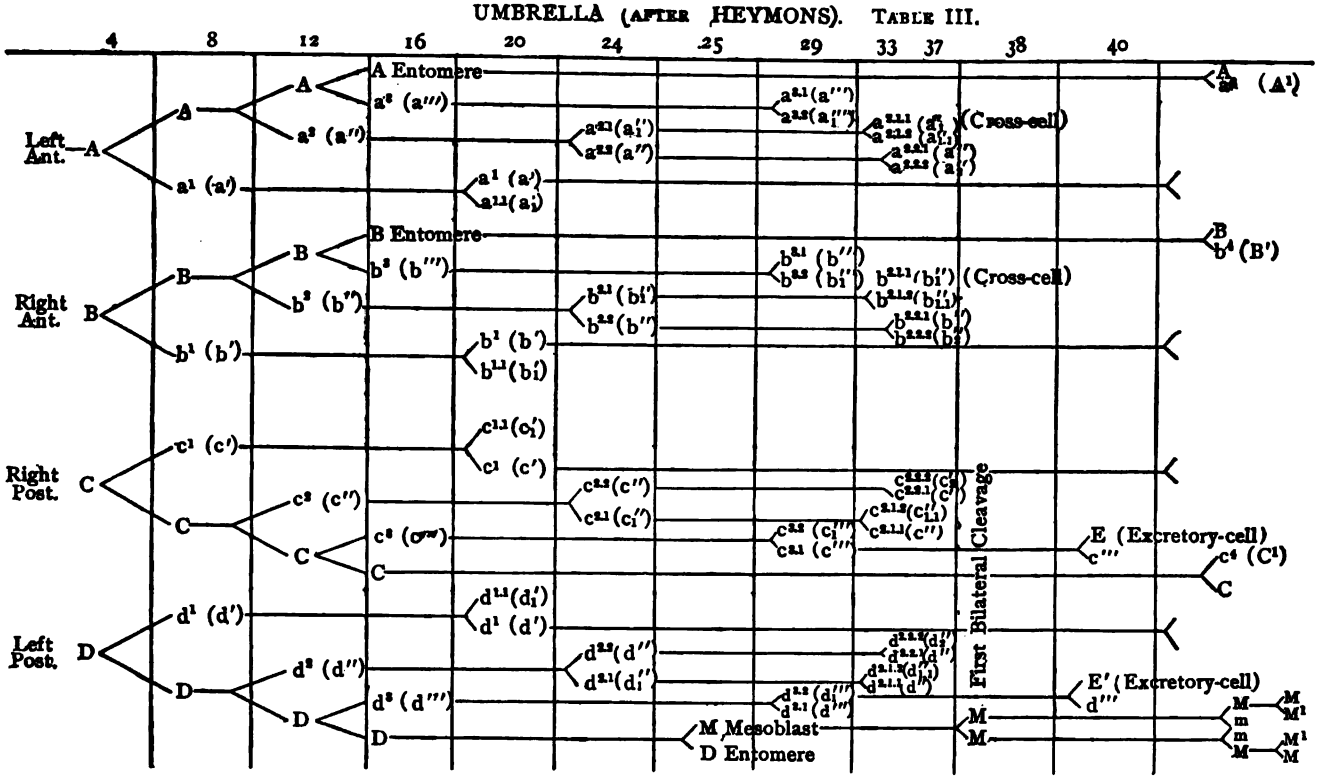
In the table of cleavages of Umbrella (Table III) I have not included all of the details described by Heymons after the forty-cell stage. Heymons has followed the cleavage cell by cell up to about 100 cells. The table given does not as a consequence give a correct impression of the immense detail of Heymons' work. The stages described after the 40-cell stage are: the 44-, 47-, 51-, 55-, 57-, 63-, 67-, 69-, 75-, 81-, 91-cell stages. The cleavage of the entomeres was followed far beyond these stages. It is interesting to notice the almost purely arithmetical progression in the increase in number of the cells after the four-cell stage. The disturbances in the regularity of this law are due to precocious separation of important blastomeres. *E.g.*, 24 to 25 cells due to formation of the mesoblast (*v.* table); 37 to 38 due to bilateral cleavage of mesoblast; 38 to 40 another bilateral cleavage, separating excretory cells *E* and *E'*; 55 to 57 bilateral divisions of Mesoblasts. A better illustration of Rabl's too-inclusive law could not be desired. The cleavage in Unio (*v.* table, p. 33) shows that precocious segregations may entirely destroy the orderly progression. The same table up to the twenty-five-cell stage at least will do equally well for Crepidula. Conklin has followed the cleavage very much farther, but has not yet published the details in such a way that they can be tabulated.

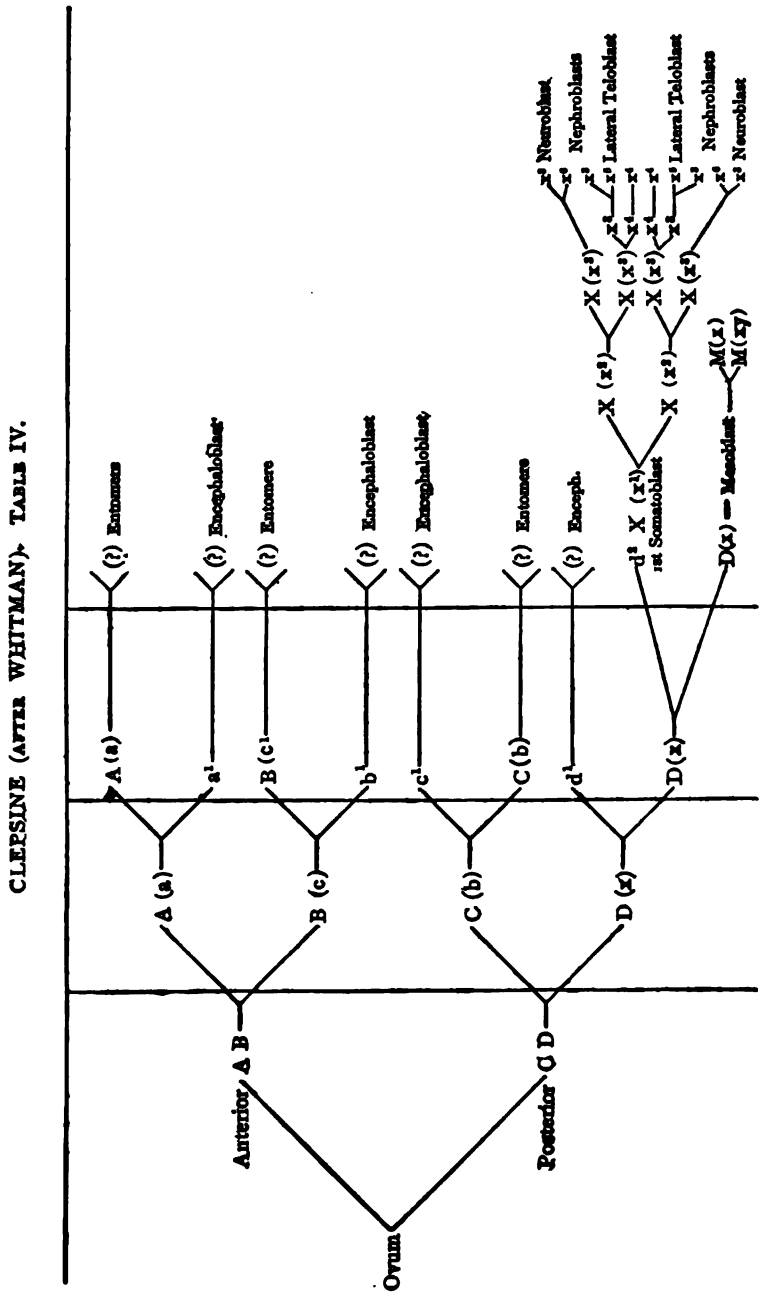
As Wilson has pointed out, it is probable that v. Wistinghausen (Table VI) has overlooked one cleavage of the posterior macromere so that the mesoblast would arise from the fourth, not from the third cleavage of this cell.


NERITINA (AFTER BLOCHMANN). TABLE I.
4
8
12
16
24
28
36
A (b) Entomere
B (a) Entomere
C (d) Entomere
D (c) Entomere
M Mesoblast
Trochoblast
A (b)
B (a)
C (d)
D (c)

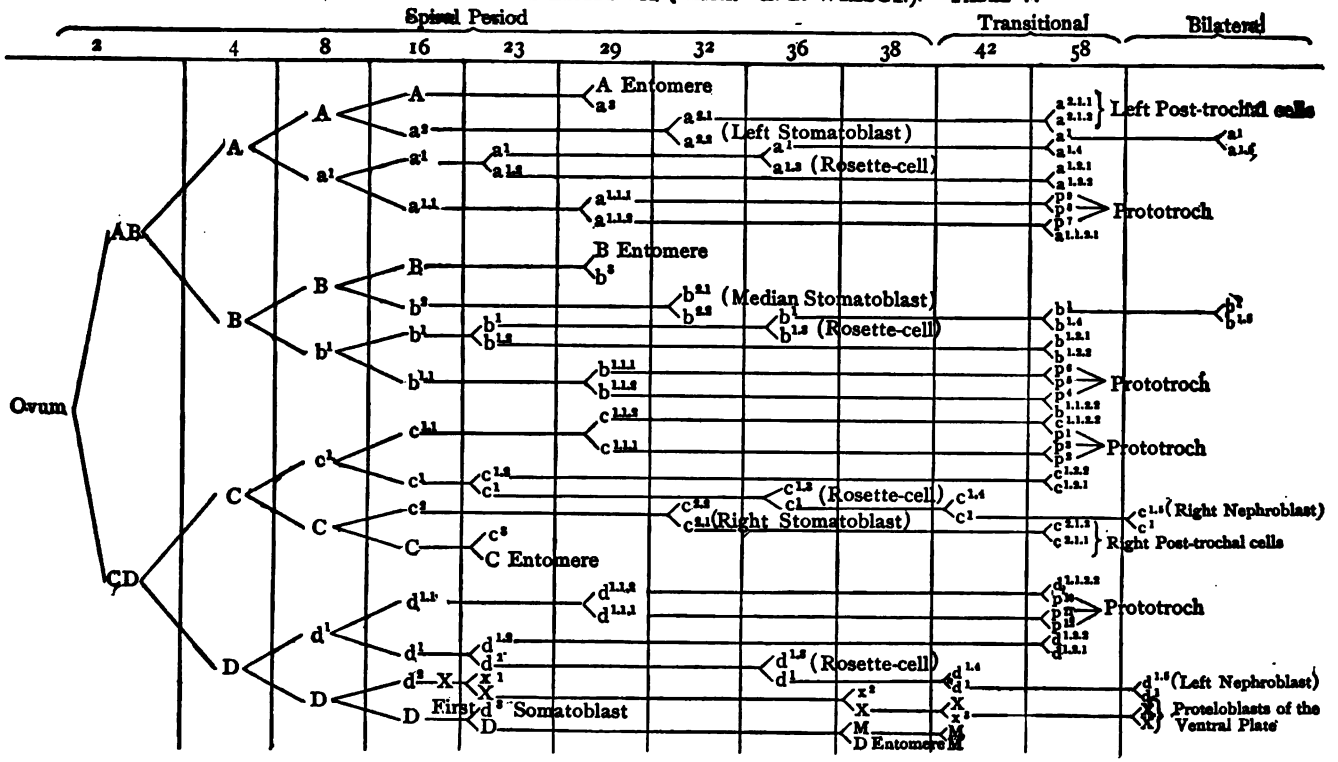
PLANORBIS (AFTER RABL). TABLE II.

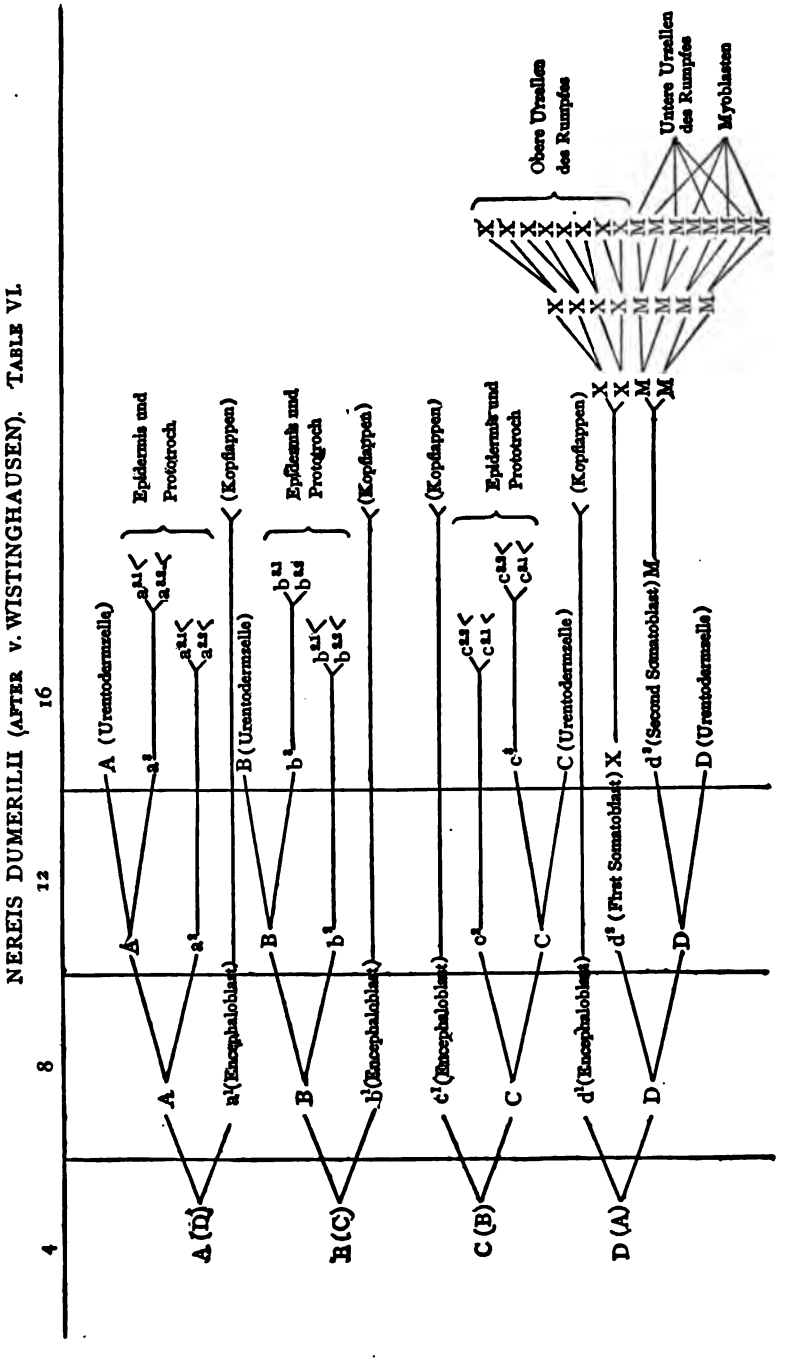



UMBRELLA (AFTER HEYMONS). TABLE III.
4
8
12
16
20
24
25
29
33
37
38
40
Left Ant.
Right Ant.
Right Post.
Left Post.
A Entomere
B Entomere
(Cross-cell)
First Bilateral Cleavage
E (Excretory-cell)
E' (Excretory-cell)
M Mesoblast
D Entomere

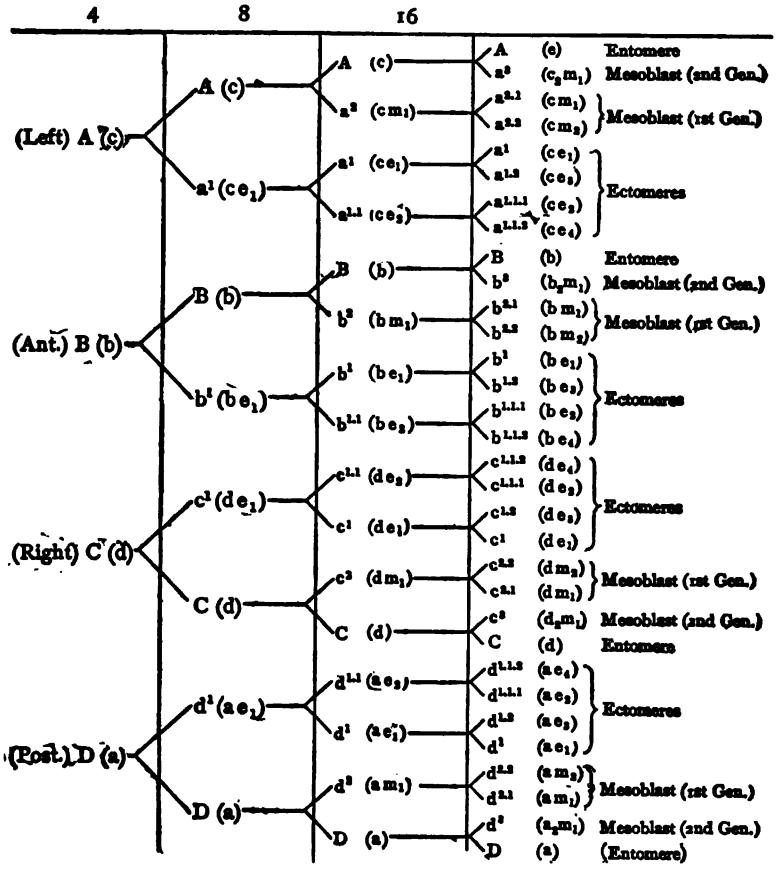

CLEPSINE (AFTER WHITMAN). TABLE IV.
Ovum
Anterior A B
Posterior C D
A (a)
B (c)
C (b)
D (x)
A (a)
a¹
B (c¹)
b¹
c¹
C (b)
d¹
D (x)
(?) Entomere
(?) Encephaloblast
(?) Entomere
(?) Encephaloblast
(?) Encephaloblast
(?) Entomere
(?) Encph.
d² X (x¹)
1st Somatoblast
D(x) = Mesoblast
X (x²)
X (x²)
X (x²)
X (x²)
M(x)
M(xy)
x² Neuroblast
x² Nephroblasts
x² Lateral Teloblast
x² Lateral Teloblast
x² Nephroblasts
x² Neuroblast

NEREIS LIMBATA (AFTER E. B. WILSON). TABLE V.




NEREIS DUMERILII (AFTER v. WISTINGHAUSEN). TABLE VI.
4
8
12
16
A (Urentodermzelle)
Epidermis und Prototroch
(Kopflappen)
B (Urentodermzelle)
Epidermis und Prototroch
(Kopflappen)
(Kopflappen)
Epidermis und Prototroch
(Kopflappen)
C (Urentodermzelle)
X
M
d² (Second Somatoblast) M
D (Urentodermzelle)
Obere Urzellen des Rumpfes
Untere Urzellen des Rumpfes
Myoblasten
A
a¹ (Encephaloblast)
B
b¹ (Encephaloblast)
C
c¹ (Encephaloblast)
D
d¹ (Encephaloblast)
d² (First Somatoblast) X
A (D)
B (C)
C (B)
D (A)

DISCOCOELIS (AFTER LANG). TABLE VII



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REFERENCE LETTERS.

a.m.	Adductor muscle.	*mes. tel.*	Teloblasts of mesoderm.
ap.	Apical pole.	*mes.*	Mesoderm.
ap. n.	Apical nucleus of thread-gland.	*mk.*	Micropyle.
bp.	Blastopore.	*o.p.*	Oral plate.
c.g.	Cerebral ganglia.	*p.t.g.*	Protoblast of thread-gland.
ent.	Entoderm.	*s.*	Shell.
f.	Foot rudiment.	*s.g.*	Shell-gland.
k.v.	Cells of head vesicle.	*s.h.*	Sensory hairs.
k.a.	Kidney anlage.	*t.g.*	Thread-gland.
l.p.	Lateral pits.	*v.p.*	Ventral plate.

A Left macromere.
B Anterior macromere.
C Right macromere.
D Posterior macromere.
a^1, b^1, c^1, d^1, $a^{1.1}$, *etc.*, First generation of ectomeres.
a^2, b^2, c^2, d^2, $a^{2.1}$, *etc.*, Second generation of ectomeres.
a^3, b^3, c^3, d^3, *etc.*, Third generation of ectomeres.
$X = d^2$ First somatoblast.
$M = d^4$ Second somatoblast.
$Y = a^{2.2}$ Larval mesoblast.

DESCRIPTION OF PLATES.

All figures were drawn with a camera lucida under a magnification of 275 diameters, except where otherwise stated. With two exceptions (Figs. 3 and 8) no attempt has been made to show the actual appearance of the segmenting ovum. A light, uniform shading has been adopted throughout for the sake of clearness. This of course does not hold for the other figures of Plates V and VI.

EXPLANATION OF PLATE I.

FIG. 1. Egg-plates of Unio complanata magnified three times.
(*a*) The flat surface.
(*b*) From the side.

FIG. 2. Part of Fig. 1 (*a*) more highly magnified.

FIG. 3. Ovum within vitelline membrane; polar globules have been formed opposite to the micropyle (*m k.*).

FIG. 4. Early two-cell stage.

FIG. 5. Later two-cell stage in outline.

FIG. 6. Preparatory to three-cell stage from the upper pole.

FIG. 7. Three-cell stage from the upper pole.

FIG. 8. Three-cell stage from the lower pole. Actual appearance of egg shown.

FIG. 9. Early four-cell stage from the upper pole; cross-furrow small. I–I, first cleavage-furrow; II–II, second cleavage-furrow.

FIG. 10. Later four-cell stage from the upper pole; cross-furrow large. I–I, first cleavage-furrow; II–II, second cleavage-furrow.

FIG. 11. The four-cell stage from in front.

FIG. 12. The five-cell stage from the upper pole. This egg is remarkable inasmuch as *A*, not *D*, is the largest of the macromeres. The lower ends of the spindles in *B* and *C* are outlined more faintly than the upper ends.

FIG. 13. Six-cell stage from the upper pole.

FIG. 14. Six-cell stage from in front.

FIG. 15. Eight-cell stage from above.

EXPLANATION OF PLATE II.

FIG. 16. Eight-cell stage from the right side. Lower pole rolled up a little.

FIG. 17. Passage to nine-cell stage from behind. Formation of first somatoblast, $d^2 = X$.

FIG. 18. Anterior view of the same egg.

FIG. 19. Ten-cell stage from the right side. Formation of the second generation of ectomeres.

FIG. 20. Thirteen-cell stage from the upper pole. d^1 of the first generation of ectomeres has divided. The large cells of the second row are the members of the second generation of ectomeres.

FIG. 21. The same egg from the right side, showing the arrangement of the macromeres also.

FIG. 22. Seventeen-cell stage from the upper pole. All of the first generation of ectomeres have divided. x^1 is the seventeenth cell.

FIG. 23. The same egg from the vegetative pole. The spindle in D is for the separation of d^3 to the left.

FIG. 24. Eighteen-cell stage from the upper pole and partly from in front. Spindles in a^2, b^2, c^2, and d^2 (*cf.* Fig. 25).

FIG. 25. The same egg from the left side. The spindle in a^2 separates $a^{2.2}$ — the larval mesoblast.

FIG. 26. Eighteen-cell stage directly from above.

FIG. 27. The same egg from the lower pole.

EXPLANATION OF PLATE III.

FIG. 28. Twenty-two-cell stage from the upper pole; rolled somewhat to the left.

FIG. 29. The same egg from the left side. Larval mesoblast—*Y*—partly overgrown by x^2 and d^3 (*cf.* Pl. II, Fig. 25).

FIG. 30. Slightly later stage from the upper pole. Second division of d^1.

FIG. 31. Formation of x^3. Upper pole.

FIG. 32. Same egg. Lower pole. Formation of third generation of ectomeres.

FIG. 33. Upper pole. Formation of x^3.

FIG. 34. Lower pole. Formation of third generation of ectomeres.

FIGS. 35–38. Four views of the same egg after the formation of x^3 and the third generation of ectomeres. Twenty-seven-cell stage.

FIG. 35. Upper pole.

FIG. 36. Anterior view; in part from above.

FIG. 37. Direct view from in front.

FIG. 38. Lower pole.

FIG. 39. The next stage from below. The indicated cleavage of *D* completes the separation of the germinal layers. x^4 in process of formation.

Mau

EXPLANATION OF PLATE IV.

FIG. 40. Thirty-one-cell stage. Lower pole.

FIG. 41. Lower pole. Division of $c^{2.1}$.

FIG. 42. Lower pole. Division of $b^{2.1}$, $b^{2.2}$, $c^{2.2}$ and d^2. Thirty-seven-cell stage.

FIG. 43. The same stage from in front.

FIG. 44. Equal division of *X*. First bilateral cleavage. Notice relations of x^1, x^2, x^3, and x^4 to *X*. View from behind.

FIG. 45. Lower pole at the time of the bilateral division of *M*.

FIG. 46. View from behind. Formation of x^5.

FIG. 47. Same stage from the right side.

FIG. 48. Upper pole of stage of Fig. 44. Division of a^1 and $a^{2.1}$.

FIG. 49. Upper pole. Division of c^1 and a^1.

FIG. 50. Upper pole. Sixteen cells of the first generation of ectomeres.

FIG. 51. Slightly later stage. Division of $d^{1.2}$. Stage of Fig. 45. Fifty cells.

EXPLANATION OF PLATE V.

FIG. 59. Left side of embryo of over fifty cells, showing inclusion of *Y* and formation of y^2.

FIG. 60. Superficial budding of the mesoblasts.

FIG. 61. Horizontal optical section of stage with four cells of the shell-gland. The larval mesoblast — *Y* — is passing into the segmentation cavity. Notice the asymmetry of the primary mesoblasts — *M*.

FIG. 62. Transverse section (actual) of a later stage, showing bilateral cleavage of *Y*.

FIG. 63. Optical section in the same plane as Fig. 61. Divisions of *M* and *Y*.

FIG. 64. View of gastrula from dorsal surface. The large cells are the rudiment of the shell-gland.

FIG. 65. Gastrula from lower pole. Entoderm in sepia.

FIGS. 66–67. Successive sagittal sections of the stage of Figs. 64, 65, and 72. Fig. 66 passes a little to one side of the middle line; Fig. 67 directly in the median line. See line of section in Fig. 65.

FIG. 68. Section of same stage through shell-gland and blastopore. See line of section in Fig. 65.

FIG. 69. Median sagittal section after the invagination of the shell-gland.

FIG. 70. Horizontal section of the same stage as Fig. 69.

FIG. 71. The six cells from the region of the thread-gland. The central cell is the protoblast of the gland. From the same stage as Fig. 69.

FIG. 72. Gastrula from the side.

FIG. 73. Median sagittal section of larva considerably younger than stage of Fig. 79.

FIG. 74. Part of Fig. 73 more highly magnified (Leitz Hom. oel-immers., $\frac{1}{12}$). Compare apical nucleus of thread-gland with nuclei on either side of aperture; compare, also, with Fig. 71.

FIGS. 75–78. Transverse sections from before-backward of the stage of Fig. 73. For region of sections, *v.* Fig. 73.

FIG. 79. Young larva of Unio from the right side. *Cf.* apical nucleus with *ap. n.* of Figs. 73 and 74. *Extremely fine* cilia of ventral plate not represented.

FIG. 80. The same from in front.

FIG. 81. Arrangement of nuclei (× 500) around opening of thread-gland of Fig. 80. (*Cp.* Fig. 71.)

EXPLANATION OF PLATE VI.

FIG. 82. Embryo slightly older than Fig. 79; seen from the ventral surface. Invagination of larval mantle begun.

FIG. 83. Obliquely sagittal section of stage of Fig. 82; *v.p.*, ventral plate.

FIG. 84. Median sagittal section of same stage.

FIG. 85. Ventral half of transverse section through stage of Fig. 82. Shell not drawn in.

FIG. 86. Horizontal section through ventral wall of same stage.

FIG. 87. Section slightly dorsal to Fig. 86 (*v.* Fig. 84 for plane of section).

FIG. 88. Median sagittal section of the young glochidium of Unio complanata.

FIG. 89. Transverse section of the same stage, passing through the intestine.

FIG. 90. Same series; three sections (7½ μ) in front.

FIG. 91. Horizontal section of same stage, showing opening of thread-gland into mantle cavity.

FIG. 92. February Glochidium of Anodonta, anterior view; only a mere fraction of thread drawn.

FIG. 93. Ventral view of same; shell gaping.

FIGS. 94–97. Four transverse sections from stage of Fig. 93. Sections asymmetrical, passing further forward on left side.

FIG. 94. Through cerebral ganglia.

FIG. 95. Three sections posterior to Fig. 94; *oes.*, rudiment of oesophagus.

FIG. 96. Five sections posterior to Fig. 94.

FIG. 97. Six sections posterior to Fig. 94.

(Sections 7.5 μ thick.)

THE EMBRYOLOGY OF THE UNIONIDAE

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A THESIS

FOR THE

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